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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: January 12, 2005

SUBJECT: Cancer Assessment Review Committee Meeting on Simazine

FROM: Jessica Kidwell
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Simazine prepared by John Liccione.

A meeting to re-review the carcinogenicity classification of this chemical is scheduled for Wednesday, February 16, 2005 at 10 am in Room 813, CM2.

Addressees:

K. Baetcke
L. Brunsman
W. Burnam
M. Copley
V. Dellarco
K. Farwell
A. Khasawinah
J. Kidwell
J. Liccione
N. McCarroll
T. McMahon
W. Phang
J. Pletcher
E. Rinde
J. Rowland
L. Taylor
Y. Woo

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

HED DOC. NO 014431

MEMORANDUM

DATE: December 13, 2000

SUBJECT: Atrazine: Evaluation of Carcinogenic Potential

FROM: Karl P. Baetcke, PhD
Vicki Dellarco, PhD
Health Effects Division (7509C), Office of Pesticide Programs

THROUGH: William Burnam, Chairman, Cancer Assessment Review Committee
Health Effects Division (7509C), Office of Pesticide Programs

TO: Sanjivani Diwan, PhD
Executive Secretary, Cancer Assessment Review Committee
Registration Branch 4
Health Effects Division (7509C), Office of Pesticide Programs

This memorandum contains the conclusions from the seventh Health Effects Division (HED) Cancer Assessment Review Committee (CARC) meeting (December 13, 2000) subsequent to the sixth CARC meeting held in November 2000 (Memorandum, From Roger Hawks to Catherine Eiden, November 1, 2000). The conclusions from the November 1, 2000 meeting were considered provisional pending receipt and review of the written comments from the June 27th to 29th, 2000 FIFRA Scientific Advisory Panel (SAP) meeting which convened to consider a preliminary hazard and dose-response assessment for atrazine prepared by HED[1]. The final report of the June 27, 2000 SAP meeting is now available[2]. Thus, the purpose of the December 13, 2000 CARC meeting was to consider and revise, if necessary, and finalize provisional conclusions of the November 1, 2000 CARC meeting.

[1]see http://www.epa.gov.scipoly/sap/2000/june27finalparta_atz.pdf
[2]see http://www.epa.gov.scipoly/sap/2000/june27/finalatrazine.pdf

CARC members present:

Lori Brunsman _____

Joycelyn Stewart _____

Clark Swentzel _____

Mike Ioannou _____

Vicki Dellarco _____

Karl Baetcke _____

Virginia Dobozy _____

Marion Copley _____

Bill Burnam _____

Linda Taylor _____

_____

Others present:
Cathy Eiden
(HED - nonvoting) _____

EXECUTIVE SUMMARY

At a meeting of the CARC held on December 13, 2000, atrazine was classified as "Not Likely To Be Carcinogenic To Humans" in accordance with the draft Guidelines for Carcinogen Risk Assessment (July, 1999). This decision was based on the information discussed below.

Atrazine is associated with mammary and pituitary tumors in female Sprague-Dawley (SD) rats, but not in male SD rats, or either sex of Fischer 344 (F-344) rats or CD-1 mice. Mutagenic and estrogenic activity do not appear to play a significant role in atrazine-associated carcinogenicity. Biological plausibility has been established for the mode of carcinogenic activity of atrazine. The rat cancer mode of action (MOA) involves a process consisting of modulation of the gonadotrophin releasing hormone (GnRH) pulse, attenuation of pituitary releases of luteinizing hormone (LH), and alteration of ovulatory cycles, expressed as constant estrus, which leads to prolonged exposure of mammary and pituitary tissues to estrogen and prolactin, and development of tumors in response to the prolonged hormone exposures. This MOA essentially accelerates the normal aging process in female SD rats. It would be expected to be operative in other rat strains with a similar reproductive aging process (e.g. Long Evans and Wistar). Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen and prolactin) that is found in SD rats is not expected to occur in humans. Instead, humans respond to reduced LH by having reductions in estrogen and prolactin. Although possible associations between atrazine exposure and non-Hodgkins lymphoma (NHL) and ovarian cancer have been reported in a few epidemiology studies, there is no supporting evidence or a sound argument of biological plausibility that these cancers may result from exposure to atrazine. Also, the lack of multiple confirming studies indicates that the human investigations by themselves do not make a strong case for an association between atrazine exposure and human cancer.

I. INTRODUCTION

At the meeting of the CARC held on December 13, 2000, the final report of the Scientific Advisory Panel (SAP Report No. 2000-05) was considered along with the provisional conclusions reached in the previous meeting of the CARC (November 1, 2000). The major conclusions of the SAP were as follows:

1. High doses of atrazine cause an increased incidence and earlier appearance of mammary adenomas and carcinomas in female SD rats but not in female F-344 rats, male SD or F-344 rats, or CD-1 mice of either sex.

2. Atrazine's MOA for the development of mammary tumors has been demonstrated. The SAP pointed out the uncertainties in the MOA but concluded that the weaknesses and limitations have been adequately addressed and are not sufficient to raise doubt about the overall MOA.

3. Regarding the question of relevance of the MOA in rats to humans, the SAP concluded:

a) There are similarities in the control of the hypothalamic-pituitary-ovarian axis between humans and rats but there are important differences. The MOA for mammary tumors in SD rats

is an acceleration of the reproductive aging process in which decreased LH levels lead to prolonged exposure of mammary tissue to estrogen and prolactin. In contrast, reproductive aging (menopause) in human females is characterized by low levels of estrogen and high levels of LH and follicle stimulating hormone (FSH).

b) There was some concern about epidemiology studies demonstrating a possible increased risk of NHL and ovarian cancer associated with atrazine exposure. However, the Panel concluded there was not a strong association due to the lack of multiple studies and some inconsistencies in the reported studies.

c) The Panel concluded that hypothalamic amenorrhea (HA) and polycystic ovarian syndrome (PCOS), anovulatory conditions in human females proposed by EPA as possible correlates to the reproductive effects of atrazine in rats, present much different endocrine profiles than age-related persistent estrus in SD rats.

4. It was the consensus of the SAP that atrazine should be classified as either "Not Likely to be Carcinogenic To Humans" or "Not Enough Information to Classify". The Panel also concluded that the MOA for atrazine carcinogenicity is not applicable to developing fetuses and children.

A preliminary hazard and dose-response assessment that was presented to the SAP (June 27, 2000) concluded that atrazine should be classified as "Likely To Be Carcinogenic To Humans." The "Likely" cancer classification was proposed because there is some evidence in the literature that CNS-acting drugs, like atrazine, may disrupt the GnRH and LH pulses and lead to disruption of the menstrual cycle in primates and humans. Further, it was thought that conditions of anovulation in humans, although in several respects dissimilar to atrazine's mode of action in the SD female rat, raised uncertainties about the possible endocrine imbalance by this CNS mode of action. Therefore, it was proposed to the June 27th SAP that human relevance should be presumed. However, as noted above, the June 27th SAP expressed the view that the mode of carcinogenic action of atrazine is not expected to be operative in humans and that atrazine should not be classified as a "Likely" human carcinogen but that "it would be more appropriate to classify atrazine as either "Unlikely To Be a Human Carcinogen" or "Not Enough Information To Classify." At the November 1, 2000 CARC meeting, the view of the SAP was discussed and atrazine was reclassified, subject to review of the final SAP report, as "Not Likely To Be Carcinogenic To Humans." Below is a reconsideration of the November CARC conclusions in light of the SAP final report.

II. EVALUATION OF CARCINOGENICITY

In reaching a final decision on the carcinogenicity classification for atrazine, the committee considered the following information.

1. Data demonstrating an increased incidence and decreased time to onset of mammary and pituitary tumors in female SD rats, but not in male SD rats or F-344 rats or CD-1 mice of either sex.

2. Data on the proposed MOA associated with the carcinogenesis seen in female SD rats

following atrazine exposure.

3. Comments provided in the final report of the SAP meeting of June 27, 2000 regarding the relevance of the MOA established for rat carcinogenicity to humans.

4. Evidence that mutagenicity and direct estrogenic activity do not play a significant role in atrazine-associated carcinogenicity.

5. Results of epidemiology studies that suggest an association between atrazine exposure and carcinogenicity in humans.

III. COMMITTEE'S ASSESSMENT OF THE WEIGHT OF EVIDENCE

The following factors were considered in evaluating the weight of evidence.

A MOA has been established for these mammary and pituitary tumors in female SD rats that is unlikely to be operative in humans. Previously the CARC classified atrazine as a "Likely" carcinogen and the draft document presented to SAP June 27, 2000 reflected this opinion. This classification assumed that a pair of human models of anovulatory conditions associated with aberrant GnRH pulses (PCOS and HA) were models of the above-described rat MOA in humans. The deliberations at the June SAP meeting clearly reflected the SAP's view that these two human models were not appropriate for comparison to the SD rat model and did not establish the human relevance for the proposed mode of action. GnRH pulse modulation of pituitary releases of LH is a central driver of ovulation in the SD female rat, and atrazine is essentially accelerating the aging process of the CNS control of ovulation, which leads to a constant state of estrus (anovulation), and prolonged exposure to estrogen and prolactin. As noted by the SAP, although there are certain similarities in the control of the hypothalamic-pituitary- ovarian axis between humans and rats in that the hypothalamus can play a key regulatory role in primates, there are fundamental differences. Unlike the SD rat, CNS modulation is not the driving factor on human GnRH and LH releases. The EPA preliminary atrazine hazard and dose-response assessment wrongly assumed that an increase in estrogen could result from an attenuation of the LH release in humans. Although human conditions of anovulation are associated with aberrant GnRH and LH pulsatile releases and even if atrazine induced anovulation in humans like in the SD rat, there is no evidence for the potential of an unopposed estrogen condition in humans that would lead to tumor development. It appears that in humans when LH is low, such as in HA, a state of low serum estrogen is found, not elevated or prolonged estrogen exposure. There is no known cancer risk associated with HA patients, albeit they are at risk to a number of other clinical conditions (e.g., osteoporosis, heart disease, infertility). Another condition of anovulation, PCOS, is also not a good model for atrazine cancer MOA in SD rats. The etiology of PCOS is multi factorial, and LH secretion is elevated due to increased synthesis of androgen and its conversion to estrogens. Although atrazine might cause adverse effects on hypothalamic-pituitary function in humans, the hormonal environment conducive to tumor development (i.e., elevated or prolonged exposure to estrogen or prolactin) that is found in SD rats is not present in humans. Therefore, it is unlikely that atrazine's mode of cancer action in SD rats is operative in humans. The CARC

agreed with the view reflected in the written comments of the June 2000 SAP review.

The human epidemiology database does not provide sufficient evidence to associate atrazine with human cancer of any tissue. The SAP report contains a discussion of issues regarding the Agency's evaluation of the human epidemiology data on atrazine and recommendations for further analyses of the data. Despite some of the short-comings pointed out by the panel, the panel stated that the summary paragraph on the evaluation of the human epidemiology in the Agency's assessment document should be revised to:

> "To summarize, there are a few epidemiological studies that suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer. However, lack of multiple studies available indicates that the human studies by themselves do not make a strong case for an association."

On closer evaluation since the June SAP meeting, the CARC agreed with the SAP that the human studies "by themselves do not make a strong case" for an association between atrazine exposure and a cancer risk. Although possible associations between atrazine exposure and NHL and ovarian cancer are reported, there is no supporting evidence or a sound argument of biological plausibility that these cancers may result from exposure to atrazine. Several two-year bioassays with atrazine in SD and F-344 rats, and CD-1 mice failed to show evidence of an increased incidence of ovarian tumors or lymphomas. Furthermore, ovarian cancer is associated with frequent ovulations (not anovulation) or stimulation by FSH and LH (not suppression of LH), thus increasing their exposure to estrogens (see Fathalla, M.F., 1971, Lancet 2 (7716):163; Cramer, D.W. and Welch, W.R., 1983, J. Natl. Cancer Inst. 71(4):717-21). NHL is associated with immune dysfunction and not hormonal imbalance.

IV. CLASSIFICATION OF CARCINOGENIC POTENTIAL

Following discussion of the conclusions reached at the November 1, 2000 CARC meeting and consideration of the comments and recommendations provided by the Scientific Advisory Panel, the December 13, 2000 CARC reaffirmed the classification of atrazine as "Not Likely To Be Carcinogenic To Humans" based on the overall weight of evidence that:

1. The mode of carcinogenic activity in the female SD rat is supported by the data.

2. The mode of carcinogenic activity in the female SD rat essentially involves an acceleration of the reproductive aging process.

3. The mode of action for the carcinogenicity of atrazine is unlikely to be expressed in humans; no human conditions can be established that support a potential for atrazine to lead to carcinogenicity in humans.

4. Other modes of action are not supported by the available data and, in particular, mutagenic

and estrogenic activity do not appear to significantly contribute to atrazine's carcinogenic potential.

5. Although a few epidemiological studies suggest a possible association between atrazine (or triazine) exposure and NHL and ovarian cancer, these cancers do not appear to be plausible based on atrazine's mode of action. Therefore, the human studies by themselves do not make a strong case for an association.

The CARC agreed that a response to the SAP comments, the classification of atrazine as "not likely to be a human carcinogen", and the supporting weight of evidence for the classification should be incorporated in the atrazine hazard and dose-response assessment document when it is finalized.

FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 24 1990

MEMORANDUM 2nd

SUBJECT: Peer Review Meeting on Simazine Following SAP Review.

FROM: Henry Spencer, Ph.D. Lee Spencer 4/26/90
Review Section II
Toxicology Branch I
Health Effects Division (H7509C)

TO: Jude Andreasen
Special Review Branch
Special Review and Reregistration Division (H7508C)

The Health Effects Division (HED) Peer Review Committee met on October 25, 1989, to reconsider the evaluation of simazine following the presentation to the Scientific Advisory Panel (SAP) (Panel Meeting September 28, 1989.

A. Peer Review Committee Individuals in Attendance: (Signatures indicates concurrence unless otherwise stated).

Penelope A. Fenner-Crisp	Penelope A. Fenner-Crisp
Esther Rinde	Esther Rinde
John D. Quest	John A. Quest
Kerry Dearfield	Kerry P. Dearfield
Karl Baetcke	Karl J. Baetcke
Reto Engler	
Bill Burnam	
Bill Sette	Bill Sette
Marion Copley	Marion Copley
Julie Du	
Rich Levy	

2. Scientific Reviewer: (non-panel member responsible for data).

Henry Spencer

3. Peer Review Members in Absentia: (Members who were unable to attend the discussion, signatures indicate concurrence unless stated).

Richard Hill

Yin-Tak Woo

Robert Beliles

Marcia Van Gemert

George Z. Ghali

4. Other attendees: (Observers).

Hugh Pettigrew

Albin Kocialski

B. Conclusion:

The SAP agreed with the Peer Review of simazine classifying it as a C carcinogen based on tumors in females in one species (rat). The SAP also volunteered that a Q_1* should not be used to quantitate risk for the chemical.

However, the Peer Review Committee in attendance considered it appropriate to use the Q_1* to quantitate risk until the registrant provides data showing hormonal induction of tumors. This classification is consistent with action on similar chemicals, i.e., atrazine. In addition the Peer Review Committee recommended that the Agency ask for further mutagenicity testing to include a mouse lymphoma assay, an in vivo micronucleus assay and a cell transformation assay.

The Weight of the Evidence used to determine the classification remains the same as excerpted below from the original peer review document, dated July 31, 1989 by Esther Rinde, Ph.D.

"F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Simazine to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Simazine was not associated with increases in neoplasms when fed in the diet to CD-1 mice, at doses up to 4000 ppm. The study was considered to have been adequately conducted.

2. Simazine was associated with statistically significant increases in carcinomas of the pituitary gland (at the HDT) and mammary gland (at the mid (100 ppm) and highest dose) in the female Sprague-Dawley rat, when fed in the diet at doses up to 1000 ppm. The incidence of mammary gland tumors at the HDT was well outside the range reported for historical controls at the testing facility. The incidence of pituitary gland tumors was just outside the historical control range; however, it exceeded (considerably) the incidences reported for 6 out of 7 studies.

3. The pituitary tumors in the female rats were fatal with a possibly accelerated onset, and the mammary carcinomas also contributed to the increased mortality at the HDT, according to the study authors.

4. Although the HDT may have exceeded the MTD, the mid-dose was well below, and the mammary tumors in the female rat were statistically significantly increased at both the mid and high dose. There was also too great an interval between the mid and high doses: 100 and 1000 ppm, respectively.

5. While a hormonal influence was suggested based on the pituitary and mammary gland tumors, supporting evidence was not presented.

6. There was some evidence of genotoxicity.

7. The mammary tumor response is consistent with that seen with other triazines. Both Atrazine and Propazine, triazines with structures closely related to Simazine, were associated with mammary gland tumors in the female rat.

8a. The incidence of kidney tubule adenomas at the HDT in the female rat, although not statistically significant, exceeded that reported for historical controls (zero) in all seven studies at the testing facility. While this tumor incidence fits the NTP definition of a "rare" tumor ($\leq$1% incidence), Dr. Slaughter offered, that based on his experience, the historical incidence of rat kidney tumors is more accurately defined as "uncommon").

8b. The incidence of kidney tubule carcinomas in male rats was less clearly defined (because of sporadic occurrences of the same tumor in control animals).

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Committee evaluated all of the evidence listed in part F (above) and concluded that Simazine should be classified as a Category C Oncogen (possible human carcinogen), based on evidence in one species, one sex. The Committee also called for a quantitative risk assessment for Simazine, quantification to be based on the mammary tumors in the female rat. The arguments for quantification were given as follows:

1a. The tumors in both the pituitary and mammary glands of the female rat were malignant.

1b. Pituitary tumors in female rats were fatal with a possible accelerated onset (analysis to be provided).

2a. Mammary tumors were statistically increased at 2 doses, albeit one above the MTD; however, there was too large a spread between the mid and high doses.

2b. Evidence of progression was suggested by mammary hyperplasia at the HDT, which correlated with tumors at that dose.

3. There was no supporting evidence for demonstrating an hormonal influence.

4. There was equivocal evidence of kidney tumors ("rare" or at least "uncommon" tumor type) in both sexes.

5. SAR was strongly supportive. Other closely-related triazines (Atrazine and Propazine) were also associated with mammary gland tumors in the female rat.

6. There was some evidence of genotoxicity."

SIMAZINE Female Rat Tumor Rates:

	Dose			
	0	10	100	1000
Mammary Gland				
Adenoma only	1/90	0/80	1/80	2/80
Fibroadenoma only	21/90	18/80	10/80	19/80
Adenoma and/or fibroadenoma only	23/90	20/80	11/80	21/80
Carcinoma	16/90	13/80	20/80	40/80
Adenoma/Fibroadenoma/ Carcinoma	39/90	33/80	31/80	61/80
Pituitary				
Adenoma only	73/90	57/80	63/79	61/80
Carcinoma	1/90	3/80	0/79	6/80
Adenoma and /or Carcinoma	74/90	60/80	63/79	67/80
Kidney Tubules				
Adenomas	0/90	0/80	0/80	2/80

7/31/89

FILE COPY

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 31 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Simazine

FROM: Esther Rinde, Ph.D. E. Rinde 6/16/89
Science Analysis and
Coordination Branch
Health Effects Division (TS-769c)

TO: James Yowell
Product Manager #23
Registration Division (TS-767c)

The Health Effects Division Peer Review Committee met on May 17, 1989 to discuss and evaluate the weight-of-the-evidence on Simazine with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope A. Fenner-Crisp	Penelope A. Fenner-Crisp
William L. Burnam	William L. Burnam
Reto Engler	Reto Engler
Edwin R. Budd	Edwin R. Budd
Marcia Van Gemert	Marcia Van Gemert
Karl Baetcke	Karl P. Baetcke
Marion Copley	Marion V. Copley
Kerry Dearfield	Kerry L. Dearfield
Richard Levy	Richard A. Levy

A. 1. Peer Review Committee (contd.)

John Quest

Esther Rinde

William Sette

Lynnard Slaughter

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Henry Spencer

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Richard Hill

Robert Beliles

George Ghali

4. Other Attendees:

Esther Saito (HED) was also present.

B. Material Reviewed:

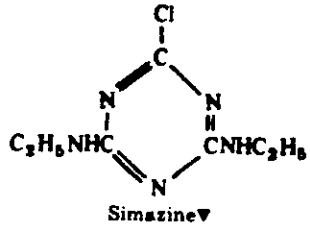
The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Henry Spencer; tables and statistical analysis by Dynamac. The material reviewed is attached to the file copy of this report.

C. Background Information:

Simazine is one of several triazine compounds which are used in agriculture as herbicides to control annual grasses and broadleaf weeds in corn, alfalfa, orchards of cherries, peaches, citrus, apples, pears and asparagus as well as ornamentals and nursery stock. Simazine is also registered for use in controlling algae in ponds. Little of the Simazine parent chemical is found as residues in food and feed crops.

Following the Data-Call-In Notice of the first Registration Standard of 1984, new chronic toxicity studies were received; these were evaluated by the Onco Peer Review Committee.

Structure of Simazine:



Simazine▼

D. Evaluation of Oncogenicity Evidence for Simazine:

1. CD-1 Mouse Oncogenicity Study

Reference: Hazelette, JR and JD Green: "Simazine Technical; 95-week Oral Toxicity/Oncogenicity Study in Mice.", April 4, 1988. Accession/MRID Number: 406144-04, Lab. Study Number: 842121. Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Simazine technical was administered in the diet to groups of 60 male and 60 female Crl:CD1(ICR)BR mice at 0 (control), 40, 1000 or 4000 ppm for 95 weeks.

There were no increases in neoplasms reported for any dosed group.

There was no evidence of a compound-related effect on survival or target organ toxicity.

The dosing was considered to be adequate for assessing the oncogenic potential of Simazine, based on body weight gain depressions of 14% in males and 19% in females seen at 1000 ppm.

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study

Reference: McCormick, CC and AT Arthur: "Simazine-Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats." , April 12, 1988. MRID Number: 406144-05. Study Number: 2-0011-09. Testing Facility: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

Simazine technical was administered in the diet to groups of 50 male and 50 female rats at 0 (control), 10, 100 or 1000 ppm for 2 years. Additional groups (30-40/sex/dose) were also treated.

> In female rats there was a statistically significant increase in mortality, and in male rats there was a statistically significant decrease in mortality, with increasing doses of Simazine.

Neoplastic lesions which occurred with statistically significant increases were reported as follows:

In female rats, there was a statistically significant dose-related trend ($p<.01$) for mammary gland carcinomas and combined adenomas/fibromas/carcinomas; however, when the shortened life-span of the female rats was included in the statistical evaluation, the incidences of carcinoma alone at both the 100 and 1000 ppm (HDT) dosage groups were statistically significantly increased as well ($p<.05$ and $p<.01$, respectively). The upper limit of the historical control incidence reported for mammary carcinoma (Table 1) was exceeded at 100 ppm, and greatly exceeded at 1000 ppm (HDT). The incidence of cystic glandular hyperplasia in the mammary gland was statistically significantly increased at the HDT, which correlates with the observed high tumor incidence at that dose.

There was a statistically significant dose-related trend for kidney tubule adenomas ($p<.05$); however (as in the case of the male rats) tumors occurred only at the HDT and the incidence (3.6%) was not statistically significant by pairwise comparison with that in the concurrent control. The incidences for adenomas and/or carcinomas reported for historical female controls (Table 1) were zero in all 7 studies (Table 1).

TABLE 1

HISTORICAL CONTROL TUMORINCIDENCE DATA
NUMBER OF TUMOR-BEARING ANIMALS - SPRAGUE-DAWLEY RATS

Submitted by Ciba-Geigy

COMPOUND	83 A	JAN 83 B	NOV 83 C	84 D	85 E	85 F	85 G
SITE: NEOPLASM			NUMBER OF NEOPLASMS				
MAMMARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	6	6	8	2	5	3	2
FIBROADENOMA	18	16	26	21	12	23	22
ADENOMA/FIBROADENOMA (COMBINED)	22	18	30	22	15	25	23
ADENOCARCINOMA	7	4	5	11	9	15	14
ALL MAMMARY TUMORS (COMBINED)	25	22	34	30	20	34	32
PITUITARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(63)	(60)	(69)	(69)	(60)	(70)	(70)
ADENOMA	52	49	55	59	49	62	62
CARCINOMA	0	2	2	2	6	2	1
ADENOMA AND CARCINOMA (COMBINED)	52	51	57	61	55	64	63
KIDNEY (MALES AND FEMALES):							
NUMBER OF SITES EXAMINED	(65/65)	(60/59)	(70/70)	(70/70)	(60/60)	(70/70)	(70/70)
	M F	M F	M F	M F	M F	M F	M F
ADENOMA	0 0	0 0	2 0	1 0	0 0	0 0	0 0
CARCINOMA	0 0	0 0	0 0	1 0	0 0	0 0	0 0
ADENOMA AND CARCINOMA (COMBINED)	0 0	0 0	2 0	2 0	0 0	0 0	0 0
ADRENAL GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	1	3	4	2	3	2	8
LIVER (MALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	0	2	0	2	10	4	1
CARCINOMA	0	1	1	6	2	1	0

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study (contd.)

In female rats, there were also statistically significant dose-related trends for adenomas, carcinomas and combined adenoma/carcinomas of the pituitary gland ($p<.01$). Pairwise comparisons were significant only for carcinomas at 1000 ppm ($p<.05$) and only when time adjusted, assuming fatal tumor context, to account for the effect of mortality disparity in the animals (the mortality in female rats was statistically significantly increased compared to controls at 100 and 1000 ppm). The incidence of pituitary gland carcinoma at 1000 ppm (HTD) only slightly exceeded the upper bound of the historical control range; however, it greatly exceeded the incidence reported in 6 out of 7 studies.

> Tables 4, 5 and 6 (from the Dynamac "..Qualitative Risk Assessment...." 10/18/88, attached) summarize these findings; a fatal tumor analysis was performed on the female rat pituitary gland tumors, as described on pg. 8 of that memo.

Historical control tumor incidence data for Sprague-Dawley rats at the testing facility are given in Table 1.

In male rats, the incidences of liver tumors were statistically significantly increased for carcinoma and for combined adenoma/carcinoma at 100 ppm and 1000 ppm (HDT), respectively ($p<.05$); however, these incidences fell within the range reported for historical controls at the testing facility.

There was also a statistically significant dose-related trend for kidney tubule carcinomas ($p<.05$), and for combined adenoma/carcinoma ($p<.01$); however, tumors occurred only at the HDT and neither the carcinoma (3%) nor the combined adenoma/carcinoma (5%) incidence was statistically significant by pairwise comparison with that in the concurrent control (2% in both cases).

> Tables 7 and 9 (from the attached Dynamac memo) present data for the tumor incidences (adjusted for mortality differences) in liver and kidney, respectively. The rationale for the tumor analysis is presented on page 8 of the Dynamac memo.

Table 4. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Mammary Gland Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)	(27-37)
	p= 0.0689	p= 0.302	p= 0.177	p= 0.123	
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)	(7-21)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**	
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (78)	
	p< 0.0001**	p= 0.4064	p= 0.2229	p< 0.0001**	

a First Adenoma observed at 48 weeks in dose 10 ppm and the first fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.

b First carcinoma observed at 48 weeks in dose 100 ppm.

Table 5. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Kidney Tubule Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Controls
Adenoma	0/74 (0.0)	0/62 (0.0)	0/54 (0.0)	2/55c (3.6)	(all 0)
	p= 0.0062**	p= 1.0000	p= 1.0000	p= 0.1799	

c First Adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animal not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. SIMAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Pituitary Gland Tumor Rates*, Fatal Tumor Analysis and Generalized K/W Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	73/89	57/80	63/77 a	61/79	
	(82.0)	(71.2)	(81.8)	(77.2)	(80-89)
	p= 0.0033**	p= 0.9944	p= 0.0206*	p= 0.0030**	
Carcinoma	1/73	3/61	0/52	6/53 b	
	(1.4)	(4.9)	(0.0)	(11.3)	(0-10)
	p= 0.0010**	p= 0.2351	p= 0.4545	p= 0.0153*	
Adenoma Carcinoma	74/89	60/80	63/77	67/79	
	(83.1)	(75.0)	(81.8)	(84.8)	(83-92)
	p= 0.0005**	p= 0.8351	p= 0.0251*	p=0.0005**	

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the first tumor or animals not examined).

( ) Per cent

a First Adenoma observed at 35 weeks in dose 100 ppm.

b First Carcinoma observed at 72 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

Table 7. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Liver Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	1/88	2/79a	0/80	3/80	
	(1.1)	(2.5)	(0.0)	(3.8)	(0-17)
	p= 0.0824	p= 0.4594	p= 0.5238	p= 0.2752	
Carcinoma	0/88	2/79	4/80b	3/80	
	(0.0)	(2.5)	(5.0)	(3.8)	(0-9)
	p= 0.2169	p= 0.2223	p= 0.0494*	p= 0.1058	
Adenoma					
Carcinoma	1/88	4/79	4/80	6/80	
	(1.1)	(5.1)	(5.0)	(7.5)	
	p= 0.0643	p= 0.1519	p= 0.1554	p= 0.0469*	

a First Adenoma observed at 52 weeks in dose 10 ppm.

b First Carcinoma observed at 99 weeks in dose 100 ppm.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before 52 weeks or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 9. SIMAZINE SPRAGUE-DAWLEY RAT Study - Male Kidney Tubule Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000	Historical Control Range (%)
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57a (2)	(0-3)
	p= 0.0543	p= 1.0000	p= 1.0000	p= 0.5278	
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65b (3)	(0-1)
	p= 0.0332*	p= 0.1660	p= 0.1821	p= 0.2091	
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)	(0-3)
	p= 0.0056**	p= 0.1610	p= 0.1721	p= 0.1087	

a First Adenoma observed at 92 weeks in dose 1000 ppm.

b First Carcinoma observed at 78 weeks in dose 1000 ppm

c The p values for Adenomas were calculated using the Cochran-Armitage Trend Test and Fisher's Exact Test, since the Peto Prevalence method collapsed to one interval.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

D. Evaluation of Oncogenicity Evidence (contd.)

2. Sprague-Dawley Rat Oncogenicity Study (contd.)

The Committee agreed that the highest dose exceeded the MTD for female rats, based on excess deaths and body weight gain reductions of 28-45% (days 7-728). The highest dose in males appeared to have exceeded the MTD, as well, based on body weight gain reductions of 27-36% (days 7-728). The Committee also felt that there was too great an interval between the mid and high doses (100 to 1000 ppm).

E. Additional Toxicology Data on Simazine:

1. Metabolism

Simazine exhibits increased binding affinity for red blood cells following oral dosing in the rat. Almost all of orally administered Simazine was excreted in the feces and urine 96 hours after administration to rats.

2. Mutagenicity

Three mutagenicity tests have been submitted in support of the registration for Simazine. Simazine was negative in an acceptable Salmonella assay using strains TA98, TA100, TA1535, TA1537 and TA1538, with and without activation. The other two tests were found to be unacceptable: a cytogenetics assay with cultured human lymphocytes and an unscheduled DNA synthesis (UDS) assay with primary rat hepatocytes. Therefore, of the three categories of mutagenicity testing, only the gene mutation category is minimally fulfilled with data gaps in the structural chromosomal aberrations and other genotoxic effects categories.

The negative Salmonella results are consistent with published literature and results with other s-triazine herbicides. However, it is reported in the literature that Simazine is positive for gene mutations in the mouse lymphoma assay (Waters et al., Basic Life Sci 21: 275-326, 1982), the Drosophila sex-linked recessive lethal assay (ibid; also reported by the U.S. EPA Gene-Tox Program), cell transformation in Syrian hamster embryo cells (reported by the U.S. EPA Gene-Tox Program), and plant cytogenetic assays (for review see Plewa et al., Mutat Res: 136 233-245, 1984). Simazine was also reported in the literature as being negative in several other assays including yeast assays, UDS with a human cell strain, sister chromatid exchanges and a mouse micronucleus (an unacceptable protocol) (Waters et al., 1982). It was also reported negative in two assays for aneuploidy (see Dellarco et al., Mutat Res 167: 149-169, 1986).

E. 2. Mutagenicity (contd.)

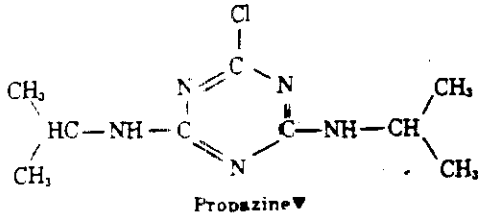
It appears then that Simazine has genotoxic potential and this would provide some support for an oncogenicity concern. Tests for submission to satisfy data gaps and to examine in more detail this genotoxic potential should include a mouse lymphoma assay, an in vivo micronucleus test and a cell transformation assay.

3. Developmental Toxicity

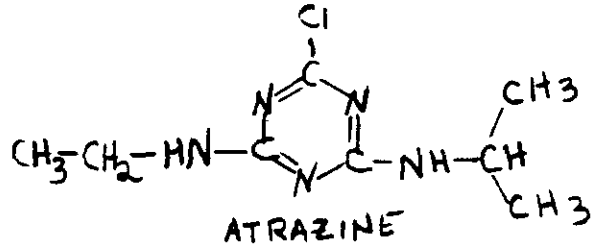
Simazine did not produce terata in the rat, when given by gavage at doses up to 600 mg/kg or in the rabbit at doses up to 200 mg/kg, by gavage; however, maternal toxicity and fetotoxicity (incomplete ossification) were observed in both species.

4. Structure-Activity Correlations

Simazine is structurally related to Atrazine, Propazine, Cyanazine, Ametryn and Prometryn. Atrazine was associated with increased mammary gland tumors in the female albino rat and was categorized as a "C(q)" oncogen by the HED Peer Review Committee. Propazine was also associated with increased mammary gland tumors in the female CD-1 rat and was categorized by the Committee as a "C" oncogen. Ametryn, Prometryn and Cyanazine have not yet been evaluated.



Propazine▼



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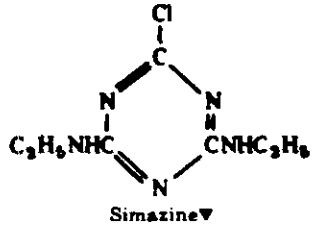
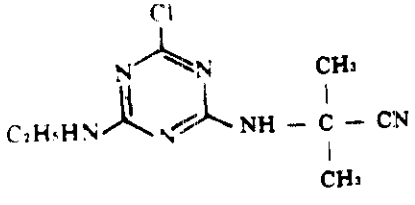
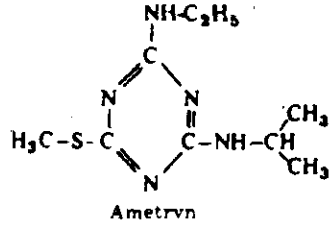


Simazine▼





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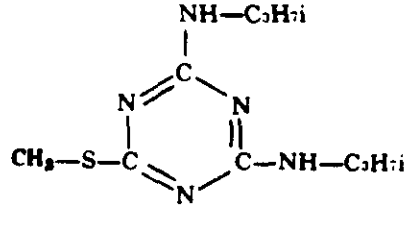

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Simazine to be of importance in a weight-of-the-evidence determination of oncogenic potential.

1. Simazine was not associated with increases in neoplasms when fed in the diet to CD-1 mice, at doses up to 4000 ppm. The study was considered to have been adequately conducted.

2. Simazine was associated with statistically significant increases in carcinomas of the pituitary gland (at the HDT) and mammary gland (at the mid (100 ppm) and highest dose) in the female Sprague-Dawley rat, when fed in the diet at doses up to 1000 ppm. The incidence of mammary gland tumors at the HDT was well outside the range reported for historical controls at the testing facility. The incidence of pituitary gland tumors was just outside the historical control range; however, it exceeded (considerably) the incidences reported for 6 out of 7 studies.

3. The pituitary tumors in the female rats were fatal with a possibly accelerated onset, and the mammary carcinomas also contributed to the increased mortality at the HDT, according to the study authors.

4. Although the HDT may have exceeded the MTD, the mid-dose was well below, and the mammary tumors in the female rat were statistically significantly increased at both the mid and high dose. There was also too great an interval between the mid and high doses: 100 and 1000 ppm, respectively.

5. While a hormonal influence was suggested based on the pituitary and mammary gland tumors, supporting evidence was not presented.

6. There was some evidence of genotoxicity.

7. The mammary tumor response is consistent with that seen with other triazines. Both Atrazine and Propazine, triazines with structures closely related to Simazine, were associated with mammary gland tumors in the female rat.

F. Weight of Evidence (contd.)

8a. The incidence of kidney tubule adenomas at the HDT in the female rat, although not statistically significant, exceeded that reported for historical controls (zero) in all seven studies at the testing facility. While this tumor incidence fits the NTP definition of a "rare" tumor (≤1% incidence), Dr. Slaughter offered, that based on his experience, the historical incidence of rat kidney tumors is more accurately defined as "uncommon").

8b. The incidence of kidney tubule carcinomas in male rats was less clearly defined (because of sporadic occurrences of the same tumor in control animals).

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Committee evaluated all of the evidence listed in part F (above) and concluded that Simazine should be classified as a Category C Oncogen (possible human carcinogen), based on evidence in one species, one sex. The Committee also called for a quantitative risk assessment for Simazine, quantification to be based on the mammary tumors in the female rat. The arguments for quantification were given as follows:

1a. The tumors in both the pituitary and mammary glands of the female rat were malignant.

1b. Pituitary tumors in female rats were fatal with a possible accelerated onset (analysis to be provided).

2a. Mammary tumors were statistically increased at 2 doses, albeit one above the MTD; however, there was too large a spread between the mid and high doses.

2b. Evidence of progression was suggested by mammary hyperplasia at the HDT, which correlated with tumors at that dose.

3. There was no supporting evidence for demonstrating an hormonal influence.

4. There was equivocal evidence of kidney tumors ("rare" or at least "uncommon" tumor type) in both sexes.

5. SAR was strongly supportive. Other closely-related triazines (Atrazine and Propazine) were also associated with mammary gland tumors in the female rat.

6. There was some evidence of genotoxicity.

5/4/89

00652

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FILE COPY

MAY 4 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Simazine.

FROM: Esther Rinde, Ph.D. E. Rinde
Manager, ONCO Peer Review
Health Effects Division (TS-769c)

TO: Addressees

Attached for your review is a package on Simazine, prepared by Henry Spencer.

A meeting to consider the classification of Simazine s scheduled for 5/17/89 at 10:00 in Room 821, CM2.

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
B. Beliles
D. Beal
J. Hauswirth
M. Van Gemert
M. Copley
J. Quest
L. Slaughter
K. Dearfield
R. Levy
W. Sette
G. Ghali
B. Fisher
H. Spencer

008512

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission of Peer Review data for evaluation of Oncogenicity of Simazine by the Peer Review Group.

FROM: Henry Spencer, Ph.D., date 4/21/89
Toxicology Branch I, (IRS), Section II
Health Effects Division, (H7509-C)

TO: Reto Engler, Ph.D., Chief,
SACB Branch
Health Effects Division (H7509-C)

THRU: Marion Copley, DVM, Section Head Marion Copley
Review Section II
Toxicology Branch I (IRS) (H7509-C)

A registration standard on Simazine was produced in 1984 and a subsequent DCI notice was transmitted to the registrant, CIBA-Geigy Corp.. New studies to evaluate the oncogenic potential of Simazine were submitted to the Agency and have been reviewed.

This submission contains the results of reviews of those new studies.

Only a chronic rat study shows an increase in female mammary tumors and male liver tumors, while the chronic mouse study appears negative for treatment related tumors. Since only one specie, the rat, appears positive for any increases in the incidence of tumor formation, the Toxicology Branch I, requests determination/confirmation whether the male and female rats both bear treatment related tumors and whether Simazine should be classified as greater than a C oncogen.

RECEIVED 4/27/89

Index of Peer Review on Simazine

Submission of Oncogenicity Data on Simazine to the Peer Review Committee

Submitted By: Henry Spencer
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., Section Head
Toxicology Branch I - IRS (H7509C)

Issue

The Peer Review Committee is requested to evaluate the oncogenicity data submitted by the registrant, Ciba-Geigy Corporation, to determine if simazine produces oncogenic effects in the test animals. Supporting data are supplied for this review.

Background

Simazine is one of several s-triazine compounds [(s) meaning symmetrical] which are used in agriculture as herbicides to control most annual grasses and broadleaf weeds in corn, alfalfa, orchards of cherries, peaches, citrus, apples, pears, and asparagus as well as ornamentals and nursery stock. Nonselective weed control in industrial settings can be achieved by using higher rates of application.

Simazine is often used in combination with other herbicides including paraquat, atrazine, and amitrole. Formulations are available as wettable powders, granulars, and liquids.

Simazine is also registered for use in controlling algae in ponds. Little of the simazine parent chemical is found as residues in food and feed crops.

The Health Effects Division (HED) of OPP has received new toxicity studies on simazine following the Data Call-In Notice of the first Registration Standard of 1984. Reviews of these studies indicate that increased incidences of mammary tumors in female rats are associated with exposure to simazine in the diet.

Toxicology Branch I (IRS) of HED submits the data reviews for evaluation and asks for Peer Review determination of the appropriate oncogenic classification of the compound.

Acute Toxicity

Simazine technical has a low acute toxicity with the rat oral LD_{50} > 5 g/kg (Toxicity Category IV) and another rabbit dermal LD_{50} > 2.0 g/kg in "limit tests."

Inhalation data in rats show that at 1.71 mg/L (the sustained maximum generated) minimal toxicity signs were evident following a 4-hour exposure (Toxicity Category IV).

Simazine is only very slightly irritating to the skin of rabbits after a 4-hour exposure (Toxicity Category IV), and is not a dermal sensitizer to guinea pigs.

Developmental Toxicity

A rat teratology study using dosages by gavage of 0, 30, 300, or 600 mg/kg exhibited maternotoxicity and fetotoxicity at 300 mg/kg and above. No malformations were reported; toxicity to the fetuses was characterized by incomplete ossification. The NOEL for the study was 30 mg/kg. Toxicity was also reported in a rabbit teratology study as nonossification of bones and reduced fetal weights; the NOEL was 75 mg/kg. Maternal toxicity was reported at 75 mg/kg. Terata formation was not evident in the study.

Subchronic (90-Day) Studies

Rodent - Technical grade simazine was fed to rats in groups of 10/sex in a powdered feed mixture at 0, 200, 2000, or 4000 ppm. Reductions in feed intake and mean body weights occurred at 2000 ppm and above. A NOEL for males based on a reduction in red blood cells (RBC) counts was less than 200 ppm (LDT). Cholesterol and inorganic phosphate levels were elevated in both sexes. Renal stones were increased at 200 ppm and above when compared to controls. The LEL was less than 200 ppm (LDT) (Attachment 1).

Nonrodent - Beagle dogs in groups of 4/sex were exposed to dietary mixtures of 0, 200, 2000, or 4000 ppm simazine for 13 weeks. Clinical chemistries and hematological determinations were made midway and at termination of the study. Body weights, food and water intake, and clinical observations were also recorded.

Results - Tremors were present from 9 weeks to termination at 4000 ppm. Body weights and food consumption were reduced at 2000 ppm and above in both sexes. Reduced erythrocyte counts occurred at high doses. A NOEL was based upon reduced albumin and increased globulin levels in males. The MTD was less than 2000 ppm in both sexes based on the reduced body weights and food consumption values (Attachment 2).

Structure Activity Similarities:

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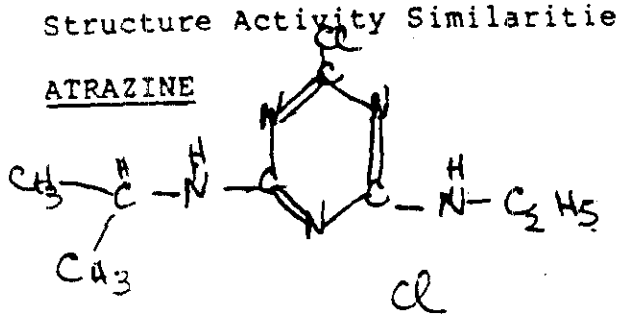
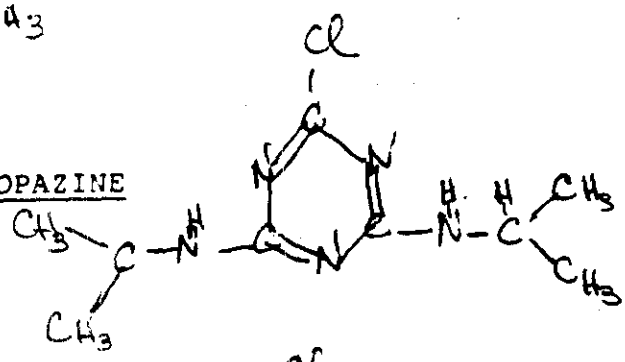


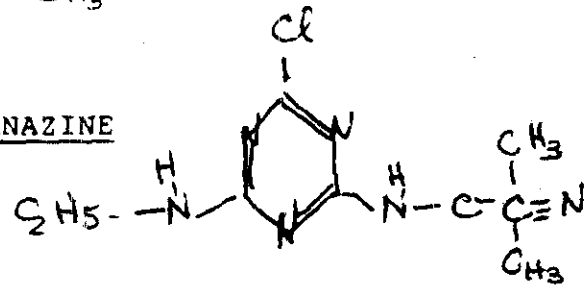
Animal Response
Increased female mammary gland tumors in the albino, rat. Peer reviewed as a "C Q*" oncogen.

PROPAZINE



Increased female CD-1 rat mammary gland tumors. Peer reviewed as a "C Q*" oncogen.

CYANAZINE



Not evaluated due to inadequate data.

Metabolism - Rats were fed 1.5 mg/kg ^{14}C ring-labeled simazine or metabolites obtained from fish fed simazine. The simazine-treated rats excreted 41 percent of the radioactivity in the feces and 49 percent in the urine. Animals fed the fish metabolites excreted 48 to 93 percent activity in the feces and 17 to 31 percent in the urine. Very small amounts of ^{14}C activity remained in the rats after 96 hours (Attachment 3).

A further study in rats indicated that simazine remained attached to RBC preferentially following oral dosing of the animals (Attachment 4).

Mutagenicity (Attachments 5, 6, 7)

Recent studies using simazine in mutagenicity evaluations have been received and provide information that an Ames assay using five doses ranging from 10 to 250 micrograms (ug)/plate was assayed at the maximum test doses possible with no evidence of mutagenic effect. Strains TA1535, TA100, TA1538, TA98, and TA1537 were used with and without S9 microsomal activation.

Structural chromosomal aberration tests were completed using human lymphocytes in vitro. The studies used both activation and nonactivation with S9 materials at concentrations of 6.25, 12.5, 25, 50, and 100 ug/mL. However, the studies were considered unacceptable because they could have been run at higher levels and posttreatment harvest time was extended beyond an optimal time period.

Unscheduled DNA repair in primary rat hepatocytes was evaluated but used too short incubation periods and presented insufficient information on dosage selection to be usable in the assay. The study was unacceptable to properly evaluate UDS.

Chronic Toxicity

Mice - Simazine was fed in the diet at levels of 0, 40, 1000, or 4000 ppm to groups of CD-1 mice containing 60 animals/sex for oncogenicity evaluation, and additional groups of 10/sex/dose for interim sacrifices at 26 and 52 weeks. Animals were observed daily and failed to show effects related to treatment at any dosage. Body weight gains were reduced at 1000 ppm and above in both sexes. Hematologic (Hct, Hgb, and RBC) changes were noted in females at 1000 ppm and above. Females appeared to be more sensitive to ingestion of simazine since most hematological effects in males were noted at 4000 ppm. Decreased organ to body weight ratios and absolute organ weights generally paralleled the lowered body weights observed in the test animals.

Neoplastic lesions were not increased significantly over values reported in control animals.

The study showed a NOEL of 40 ppm with no evidence for oncogenic potential (Attachment 8).

Rats - A chronic feeding study in Sprague-Dawley rats was used to examine simazine for oncogenic potential. Fifty rats/sex/dose were exposed to 0, 10, 100, or 1000 ppm of simazine in the diet and examined after 2 years for oncogenicity. Additional groups (30 to 40/sex/dose) were treated to determine toxicity endpoints (Attachment 9).

Survival - Male rats at the highest dose survived better than controls but females had a 20 percent survival rate compared to 34 percent in the controls.

Non-neoplastic Toxicity

Reduced body weight gains were seen in mid- and high-dose animals of both sexes. Food consumption was reduced signifi- significantly at 1000 ppm in both sexes but only occasionally at 100 ppm. Hematological parameters (Hgc, RBC, Hct) were variously depressed throughout the study at the mid- and high-dose levels in females. Clinical chemistry determinations show that glucose levels were lower at the mid and high doses in females when compared to controls. Other chemistry parameter changes were either not biologically significant or were not discernible as treatment-related.

Organ weight to body weight or brain weight changes were quite severe in the kidneys and livers of females on diets of 1000 ppm simazine. However, the great loss in body weights confounded the results. More likely, the absolute liver weights or percent of the brain weight would represent real changes in the organ weights from treatment. Therefore, the LEL for these effects was considered to be 100 ppm in females.

Neoplastic Changes (Excerpted from the TB review, Attachment 9)

Table 7. Summary of Histopathological Lesions - Male Rats

Histopathological Observation[1/]	Dose (ppm) 0	10	100	1000
Neoplastic Lesions				
Adrenal - Cortical adenoma	0/69[2/]	0/70	1/69	2/69
Kidney - Adenoma	0/70	0/70	0/70	1/70
- Carcinoma (primary)	0/70	0/70	0/70	2/70
Liver - Hepatocellular adenoma	1/70	1/70	1/70	3/70
- Hepatocarcinoma	0/70	2/70	4/70	3/70
- Combined adenoma and/or carcinoma	1/70	3/70	4/70	6/70
Thyroid - C-Cell adenoma	2/70	5/69	5/69	6/70
- C-cell carcinoma	2/70	1/69	1/69	3/70
- Combined adenoma and/or carcinoma	4/70	6/69	6/69	9/70

1/Main study only (interim sacrifice and recovery groups not included).
2/Number of rats with specified observation/total number of tissues examined.

HISTORICAL CONTROL TUMORINCIDENCE DATA
NUMBER OF TUMOR-BEARING ANIMALS - SPRAGUE-DAWLEY RATS

Submitted by Ciba-Geigy

COMPOUND	83 A	JAN 83 B	NOV 83 C	84 D	85 E	85 F	85 G
SITE: NEOPLASM			NUMBER OF NEOPLASMS				
MAMMARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	6	6	8	2	5	3	2
FIBROADENOMA	18	16	26	21	12	23	22
ADENOMA/FIBROADENOMA (COMBINED)	22	18	30	22	15	25	23
ADENOCARCINOMA	7	4	5	11	9	15	14
ALL MAMMARY TUMORS (COMBINED)	25	22	34	30	20	34	32
PITUITARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(63)	(60)	(69)	(69)	(60)	(70)	(70)
ADENOMA	52	49	55	59	49	62	62
CARCINOMA	0	2	2	2	6	2	1
ADENOMA AND CARCINOMA (COMBINED)	52	51	57	61	55	64	63
KIDNEY (MALES AND FEMALES):							
NUMBER OF SITES EXAMINED	(65/65)	(60/59)	(70/70)	(70/70)	(60/60)	(70/70)	(70/70)
	M F	M F	M F	M F	M F	M F	M F
ADENOMA	0 0	0 0	2 0	1 0	0 0	0 0	0 0
CARCINOMA	0 0	0 0	0 0	1 0	0 0	0 0	0 0
ADENOMA AND CARCINOMA (COMBINED)	0 0	0 0	2 0	2 0	0 0	0 0	0 0
ADRENAL GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	1	3	4	2	3	2	8
LIVER (MALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	0	2	0	2	10	4	1
CARCINOMA	0	1	1	6	2	1	0

Males - Male rats exhibited a significant dose-related trend for kidney tubule carcinomas ($p < .05$) or combined adenomas and carcinomas ($p < .01$). Pairwise comparisons were not significant for kidney tumors. Table 7, Summary of Lesions, uses all animals on study while the statistical evaluation uses fewer animals in the C.J. Nelson memorandum (Attachment 10). The liver tumor incidence was statistically significant in the C.J. Nelson evaluation in the Fisher's Exact test for carcinomas at 100 ppm and in the combined adenomas and carcinomas at 1000 ppm ($p < .05$). These values are considered of questionable significance when viewed in light of the historical control data submitted by Ciba-Geigy for compounds D, E, and F in the historical control tumor incidence table on page 6.

Females - Dose-related trends for the adenomas, carcinomas, or combined tumors of the pituitary gland were statistically significantly ($p < 0.03$) in each case. Pairwise comparisons were significant when examined for the effect of being a fatal tumor in the animals. Table 6 shows that increased significance at the 100 and 1000 ppm dosages occurs primarily as adenomas increase. Only at 1000 ppm is there a lifetime adjusted *increase of* carcinomas ($p < .05$).

Prior to additional statistical evaluation, no significant increases in either tumor type are seen in the TB review (Table 6).

The lack of historical control data on the onset of the pituitary tumor with time does not allow its use in this evaluation.

Mammary gland carcinomas and combined adenomas/fibroadenomas and carcinomas exhibited a significant dose-related trend ($p < .0001$) see Table 8.

However, when the shortened life span of the female rats is included in the statistical evaluation, both 100 and 1000 ppm dosage groups show significance at $p = .039$ and $p < .0001$, respectively, when compared to controls (see Attachment 9).

Data supporting an effect of simazine on tumorigenicity is the fact that cystic glandular hyperplasia was increased significantly at the HDT (1000 ppm) and only equivocally at 100 ppm when compared to controls.

Table 6. Simazine, Sprague-Dawley Rat Study--Female Pituitary Gland Tumor Rates+, Fatal Tumor Analysis and Generalized K/W Test Results (extracted from T.B. review, Attachment 9)

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	73/89 (82.0)	57/80 (71.2)	63/77[a] (81.8)	61/79 (77.2)
	p = 0.0033**	p = 0.9944	p = 0.0206*	p = 0.0030**
Carcinoma	1/73 (1.4)	3/61 (4.9)	0/52 (0.0)	6/53[b] (11.3)
	p = 0.0010**	p = 0.2351	p = 0.4545	p = 0.0153*
Adenoma/ Carcinoma	74/89 (83.1)	60/80 (75.0)	63/77 (81.8)	67/79 (84.8)
	p = 0.0005**	p = 0.8351	p = 0.0251*	p = 0.0005**

+Number of tumor-bearing animals/number of animals at risk (excluding animals that died before the first tumor or animals not examined).
() = Percent
[a]First adenoma observed at 35 weeks in dose 100 ppm.
[b]First carcinoma observed at 72 weeks in dose 1000 ppm.

Note: Significance of trend donated at control. Significance of pairwise comparison with control denoted at dose level.
*Denotes $p < 0.05$.
**Denotes $p < 0.01$.

[redacted]

Table 8. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Mammary Gland Tumor Rates and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p= 0.0689	p= 0.302	p= 0.177	p= 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (78)
	p< 0.0001**	p= 0.4064	p= 0.2229	p< 0.0001**

a First Adenoma observed at 48 weeks in dose 10 ppm and the first Fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.

b First carcinoma observed at 48 weeks in dose 100 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Due to the presence of mortality differences in both sexes of rats, the Peto prevalence test was used for incidental tumor rates to test for increasing incidence with increasing dose levels and for pair-wise differences between controls and treated rats. If the Peto prevalence method reduces to too few intervals then the Cochran-Armitage method is used to test for trends and the Fisher's exact test to test for pair-wise differences. If the tumors are considered fatal, the Thomas, Breslow, and Gart procedure is used to analyze for trends and pair-wise differences.

In the female rats, M. Copley suggested that the mammary gland adenomas and fibroadenomas be analyzed together as benign tumors, since about 50% of the rats with fibroadenomas also had carcinomas. There were no significant pair-wise comparisons or a trend noted. There was a significant dose-related trend for mammary gland carcinomas and for combined mammary gland adenomas/fibroadenomas and carcinomas ($p < 0.0001$). The incidence of mammary gland carcinomas in the 100 ppm and 1000 ppm dose groups were significantly increased ($p = 0.0392$ and $p < 0.0001$, respectively) compared to the controls. The incidence of combined mammary gland adenomas/fibroadenomas and carcinomas in the 1000 ppm dose group was significantly increased ($p < 0.0001$) compared to the controls (Table 8).

Weight-of-the-Evidence

Data on simazine, although sparse, do not indicate a strong mutagenic potential. Chronic data in rat and mice studies indicate that simazine affects body weight gains and hematological parameters in the two species. The rat showed a NOEL of 0.5 mg/kg; the mouse exhibited a NOEL of approximately 6 mg/kg.

The mouse was negative for oncogenic effects associated with exposure to simazine at up to 4000 ppm in the diet.

The effects of simazine on the mammary glands of the female rat indicate increased oncogenic potential in that sex and species. There was also a significant increase in pituitary tumors. The mechanism of tumorigenicity was not discernible from the data submitted.

The male rat data showed an increase in kidney tumors as well as liver tumors.

In summary, one species, the rat, exhibited increased incidences of female mammary tumors and pituitary tumors. The male rat exhibited a dose-related trend for increased kidney tumors and significant numbers (Fisher's Exact test) of liver tumors.

Attachments

Attachment 10 [illegible]

OCT 18, 88

EPA: 68-D8-0565
DYNAMAC No. 1-16
October 18, 1988

SIMAZINE - Qualitative Risk Assessment from a Rat Two Year Oral Chronic Toxicity and Oncogenicity Study

Caswell No. 740

APPROVED BY:

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Signature: William L McLellan (for)

Date: Oct. 20, 1988

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Signature: Richard A Levy
Date: 10-21-88

SUMMARY:

Simazine technical was fed to male and female Sprague-Dawley rats at doses of 0, 10, 100, or 1000 ppm in a 104 week chronic toxicity/oncogenicity study.

For female rats, there was a statistically significant increase in mortality with increasing doses of Simazine and mortality was significantly increased in both the 100 and 1000 ppm dose groups compared to the controls.

The incidence of mammary gland carcinomas and combined adenomas and carcinomas had a significant dose-related trend. The incidence of mammary gland carcinomas was significantly increased compared to the controls at the 100 and the 1000 ppm groups; the combined adenomas and carcinomas was significantly increased compared to the controls for the 1000 ppm group.

The pituitary gland tumors were considered fatal (reference page 1460 of the Ciba-Geigy report, attached), all three tumor groups (adenomas, carcinomas, and combined adenomas and carcinomas) showed significant dose-related trends. The incidence of pituitary adenomas and combined tumors was significantly increased compared to controls at the 100 and 1000 ppm groups; the incidence of carcinomas was significant at the 100 ppm group only.

There was a significant dose-related trend for kidney tubule adenomas.

For male rats, there was a statistically significant decrease in mortality with increasing doses of Simazine and mortality was significantly decreased in the 1000 ppm group compared to the controls.

There were no significant dose-related trends for liver adenomas, carcinomas, and combined adenomas and carcinomas. The incidence of liver carcinomas in the 100 ppm group was significantly increased compared to the controls. The incidence of combined liver adenomas and carcinomas was significantly increased compared to the controls in the 1000 ppm group. There were no significant dose-related trends or pair-wise differences for thyroid C-cell adenomas, carcinomas, and combined adenomas and carcinomas.

There was a significant dose-related trend for kidney tubule carcinomas and combined adenomas and carcinomas. There were no significant pair-wise differences for any of the kidney tubule tumors.

BACKGROUND:

Simazine technical was fed to male and female Sprague-Dawley rats at doses of 0, 10, 100, or 1000 ppm in a 104 week chronic toxicity/carcinogenicity study. Approximately 10 animals in each sex were sacrificed after 52 weeks of continuous dosing in each dose group. Only 9 animals were sacrificed in the male 10 ppm dose group and in the female 100 and 1000 ppm dose groups. This was due to deaths on study which occurred before the scheduled sacrificed since the animals to be sacrificed were selected prior to the beginning of the study. Also ten animals from the 1000 ppm group are not included in this analysis. These animals were dosed for 52 weeks and then maintained for 52 additional weeks on an untreated (control) diet. They were designated as a recovery group. A supplementary table of the results from these animals and their assigned controls was prepared (attachment 1). There were only 2 kidney tumors in the males, one adenoma in the control group and one carcinoma in the 1000 ppm group. In the females, there were 4 mammary gland adenomas in the controls and 2 in the 1000 ppm group. There was 1 mammary gland carcinoma in the controls and 4 in the 1000 ppm group. There were no pituitary gland carcinomas in either group but there were 9 adenomas in both groups.

The study was conducted by Ciba-Geigy Corporation, Pharmaceutics Division, Summit, NJ for the Ciba-Geigy Corporation. The TOX Chemical No. is 740, the MRID No. is 406144-05, and the Study No. is 2-011-09. Data was extracted from a final report dated April 12, 1988. Test animals were assigned randomly to the following dose groups:

Table 1. Experimental Design for Rat Chronic/Carcinogenicity Study

Dose (ppm)	Phase	Total Number		Time of Sacrifice 52 Weeks		Least Number of Dose Weeks
		Male	Female	Male	Female	
Control	Chronic c	10	10	10	10	52
		10	10			52 + 52-wk recovery
		20	20			104
	Carcinogenicity	50	50			104
10	Chronic c	10	10	10a	10	52
		20	20			104
	Carcinogenicity	50	50			104
100	Chronic c	10	10	10	10a	52
		20	20			104
	Carcinogenicity	50	50			104
1000	Chronic c	10	10	10	10a	52
		10b	10b			52 + 52-wk recovery
		20	20			104
	Carcinogenicity	50	50			104

a Only 9 animals were actually sacrificed in these dose groups.
b These 10 animals were excluded from analysis.
c The chronic animals were also used for hematology, biochemistry, and urinalysis.

SURVIVAL ANALYSIS:

In female rats, a statistically significant increasing trend in mortality was observed with increasing doses of Simazine ($p = 0.0036$). Mortality was significantly increased in the 100 ppm and the 1000 ppm dose group compared to the controls ($p = 0.0058$ and $p = 0.0006$ respectively). (Table 2).

In male rats, a statistically significant decreasing trend in mortality was observed with increasing doses of Simazine ($p = 0.0016$). Mortality was significantly decreased in the 1000 ppm dose group compared to the controls ($p = 0.0077$) (Table 3) .

Tests for mortality were made using the Thomas, Breslow, and Gart procedure. The earlier deaths occurred in the mid and high dose groups and the K/W test gives more weight to earlier deaths. Hence, all mortality test reported are the generalized K/W test.

TABLE 2. SIMAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Mortality Rates* and Generalized K/W Test Results

DOSE(PPM)	WEEKS 1-26	27-52	52a	53-78	79-106a	TOTAL
0.000	0/90 (0)	1/90 (1)	10/10	13/79 (16)	39/66 (59)	53/80** (66)
10.000	0/80 (0)	2/80 (2)	10/10	18/68 (26)	27/50 (54)	47/70 (67)
100.000	1/80 (1)	8/79 (10)	9/9	18/62 (29)	26/44 (59)	53/71** (75)
1000.000	0/80 (0)	5/80 (6)	9/9	21/66 (32)	31/45 (69)	57/71** (80)

TABLE 3. SIMAZINE, SPRAGUE-DAWLEY RAT Study--MALE Mortality Rates* and Generalized K/W Test Results

DOSE(PPM)	WEEKS 1-26	27-52	52a	53-78	79-106a	TOTAL
0.000	0/90 (0)	2/90 (2)	10/10	12/78 (15)	34/66 (52)	48/80** (60)
10.000	0/80 (0)	1/80 (1)	9/9	8/70 (11)	38/62 (61)	47/71 (66)
100.000	0/80 (0)	0/80 (0)	10/10	6/70 (9)	33/64 (52)	39/70 (56)
1000.000	0/80 (0)	0/80 (0)	10/10	6/70 (9)	22/64 (34)	28/70** (40)

* Number of animals that died during the interval/Number of animals alive at the beginning of the interval.

( ) Per cent

a Interim sacrifice was conducted at 52 weeks. Final sacrifice occurred at week 106.

Note: Time intervals were selected for display purposes only. Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

Table 4. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Mammary Gland Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p= 0.0689	p= 0.302	p= 0.177	p= 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (78)
	p< 0.0001**	p= 0.4044	p= 0.2229	p< 0.0001**

a First Adenoma observed at 48 weeks in dose 10 ppm and the first fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.
b First carcinoma observed at 48 weeks in dose 100 ppm.

Table 5. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Kidney Tubule Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	0/74 (0.0)	0/62 (0.0)	0/54 (0.0)	2/55c (3.6)
	p= 0.0042**	p= 1.0000	p= 1.0000	p= 0.1799

c First Adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.
* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animal not examined).
( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. SIMAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Pituitary Gland Tumor Rates*, Fatal Tumor Analysis and Generalized K/W Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	73/89	57/80	63/77 a	61/79
	(82.0)	(71.2)	(81.8)	(77.2)
	p= 0.0033**	p= 0.9964	p= 0.0206*	p= 0.0030**
Carcinoma	1/73	3/61	0/52	6/53 b
	(1.4)	(4.9)	(0.0)	(11.3)
	p= 0.0010**	p= 0.2351	p= 0.4545	p= 0.0153*
Adenoma Carcinoma	74/89	60/80	63/77	67/79
	(83.1)	(75.0)	(81.8)	(84.8)
	p= 0.0005**	p= 0.8351	p= 0.0251*	p=0.0005**

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the first tumor or animals not examined).

( ) Per cent

a First Adenoma observed at 35 weeks in dose 100 ppm.

b First Carcinoma observed at 72 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

Table 7. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Liver Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	1/88	2/79a	0/80	3/80
	(1.1)	(2.5)	(0.0)	(3.8)
	p= 0.0824	p= 0.4594	p= 0.5238	p= 0.2752
Carcinoma	0/88	2/79	4/80b	3/80
	(0.0)	(2.5)	(5.0)	(3.8)
	p= 0.2169	p= 0.2223	p= 0.0494*	p= 0.1058
Adenoma Carcinoma	1/88	4/79	4/80	6/80
	(1.1)	(5.1)	(5.0)	(7.5)
	p= 0.0643	p= 0.1519	p= 0.1554	p= 0.0469*

a First Adenoma observed at 52 weeks in dose 10 ppm.

b First Carcinoma observed at 99 weeks in dose 100 ppm.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before 52 weeks or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 8. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Thyroid C-Cell Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	2/52	7/52a	5/51	6/58
	(4)	(13)	(10)	(10)
	p= 0.3355	p= 0.0606	p= 0.1082	p= 0.0870
Carcinoma	2/34	1/31	1/36	3/45b
	(6)	(3)	(3)	(7)
	p= 0.1762	p= 0.1082	p= 0.2881	p= 0.4183
Adenoma Carcinoma	4/52	8/52	6/51	9/58
	(8)	(15)	(12)	(16)
	p= 0.1924	p= 0.1965	p= 0.2261	p= 0.1505

a First Adenoma observed at 89 weeks in dose 10 ppm.

b First Carcinoma observed at 102 weeks in dose 1000 ppm.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 9. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Kidney Tubule Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57a (2)
	p= 0.0543	p= 1.0000	p= 1.0000	p= 0.5278
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65b (3)
	p= 0.0332*	p= 0.1660	p= 0.1821	p= 0.2091
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)
	p= 0.0056**	p= 0.1610	p= 0.1721	p= 0.1087

a First Adenoma observed at 92 weeks in dose 1000 ppm.

b First Carcinoma observed at 78 weeks in dose 1000 ppm

c The p values for Adenomas were calculated using the Cochran-Armitage Trend Test and Fisher's Exact Test, since the Peto Prevalence method collapsed to one interval.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

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Peto, R., M Pike, P Day, P Gray, S Parish, J Peto, S Richard, and J Wahrendorf Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic effects in Long-term Animal Experiments. Monograph on the Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal. International Agency on Research on Cancer Monograph - Supplement 2, 311-426, 1980.

ATTACHMENT 1. SIMAZINE Female Rat Tumor Rates in rats fed 1000 ppm for 52 weeks and then allowed a 52-week recovery period compared to their matching control groups.

Tumor	Dose(ppm)	0	1000	0	1000
Mammary Gland:		FEMALES		MALES	
Adenoma and/or Fibroadenoma		4/10	2/10		
Carcinoma		1/10	4/10		
Adenoma/Fibroadenoma/ Carcinoma Combined		5/10	6/10		
Pituitary:					
Adenoma only		9/10	9/10		
Carcinoma		0/10	0/10		
Adenoma and/or Carcinoma		9/10	9/10		
Kidney Tubules:					
Adenomas		0/10	0/10	0/10	1/10
Carcinoma				1/10	0/10
Adenoma and/or Carcinoma				1/10	1/10
Liver:					
Adenoma only				0/10	0/10
Carcinoma				0/10	0/10
Adenoma and/or Carcinoma				0/10	0/10
Thyroid C-Cell:					
Adenomas				0/10	0/10
Carcinoma				0/10	0/10
Adenoma and/or Carcinoma				0/10	0/10

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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PESTICIDES AND TOXIC SUBSTANCES

Subject: Cyanazine (188C), Atrazine (63) and Simazine (740) Quantitative Risk Assessment Comparisons on Malignant Mammary Gland Tumors only in Rats. Revised Comparisons as of July, 1991.

From: Bernice Fisher, Biostatistician
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

Bernice Fisher 7/8/91

To: Karl Baetcke, Ph.D., Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Kerry L. Dearfield, Ph.D., Acting Section Head
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)
and
Reto Engler, Ph.D., Chief
Scientific Analysis & Coordination Branch
Health Effects Division (H7509C)

Kerry Dearfield 7.8.91

HED's previous estimate of cyanazine's Q_1^* of 8.8×10^{-1} was based upon malignant mammary gland tumors including fibrosarcomas. For comparative purposes with atrazine and simazine, malignant tumors including adenocarcinomas, carcinomas and carcinosarcomas only are used in the estimation of the unit risk, Q_1^*.

Animals with fibrosarcomas in the cyanazine study are excluded from the group for the estimate of Q_1^*. The reason for this exclusion is due to advice given by Dr. Brennecke (HED's consultant in pathology) that fibrosarcomas do not originate from epithelial cell tissues as do the carcinomas. The carcinosarcomas, which originate from both the epithelial and mesenchymal cell tissues, found in both the atrazine and cyanazine mammary gland malignant tumor data can be retained for the estimate of Q_1^*.

cc Kathy Pearce SRRD

Table on Estimated[+] Q_1^* $(mg/kg/day)^{-1}$ for Cyanazine, Atrazine and Simazine in Sprague-Dawley Female Rats

	Tumors in the Mammary Gland	Q_1^* $(mg/kg/day)^{-1}$ Rat	In Human Equiv.[++]
Cyanazine	Carcinosarcomas & Adenocarcinoma	1.59×10^{-1}(a)	8.4×10^{-1}(c)
Atrazine	Adenocarcinoma & Carcinosarcoma	1.72×10^{-2}(b)	9.2×10^{-2}(c)
Simazine	Carcinoma	2.25×10^{-2}(b)	1.2×10^{-1}(c)

[+] Based on results from Statox computer program
[++]Derived by the use of surface area correction - (Human Wt./Rat Wt.)$^{1/3}$

(a) Multi-Stage Model (Global86)
(b) Time-to-Tumor Multi-Stage Model (Weibull83)
(c) Cyanazine - This Q_1^* is the estimate to be used for Risk Characterization.
Atrazine - This Q_1^* is the estimate for comparative purposes only of the three chemical compounds and is not the one that is used for Risk Characterzation (actual estimate used is 2.2×10^{-1} based upon both benign and malignant mammary gland tumors).
Simazine - This Q_1^* is the estimate that has been and is still being used for Risk Characterization.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission of Peer Review data for evaluation of Oncogenicity of Simazine by the Peer Review Group.

FROM: Henry Spencer, Ph.D., 4/21/89
Toxicology Branch I, (IRS), Section II
Health Effects Division, (H7509-C)

TO: Reto Engler, Ph.D., Chief,
SACB Branch
Health Effects Division (H7509-C)

THRU: Marion Copley, DVM, Section Head
Review Section II
Toxicology Branch I (IRS) (H7509-C)

A registration standard on Simazine was produced in 1984 and a subsequent DCI notice was transmitted to the registrant, CIBA-Geigy Corp.. New studies to evaluate the oncogenic potential of Simazine were submitted to the Agency and have been reviewed.

This submission contains the results of reviews of those new studies.

Only a chronic rat study shows an increase in female mammary tumors and male liver tumors, while the chronic mouse study appears negative for treatment related tumors. Since only one specie, the rat, appears positive for any increases in the incidence of tumor formation, the Toxicology Branch I, requests determination/confirmation whether the male and female rats both bear treatment related tumors and whether Simazine should be classified as greater than a C oncogen.

RECEIVED 4/27/89

Index of Peer Review on Simazine

Submission of Oncogenicity Data on Simazine to the Peer Review Committee

Submitted By: Henry Spencer
Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., Section Head
Toxicology Branch I - IRS (H7509C)

Issue

The Peer Review Committee is requested to evaluate the oncogenicity data submitted by the registrant, Ciba-Geigy Corporation, to determine if simazine produces oncogenic effects in the test animals. Supporting data are supplied for this review.

Background

Simazine is one of several s-triazine compounds [(s) meaning symmetrical] which are used in agriculture as herbicides to control most annual grasses and broadleaf weeds in corn, alfalfa, orchards of cherries, peaches, citrus, apples, pears, and asparagus as well as ornamentals and nursery stock. Nonselective weed control in industrial settings can be achieved by using higher rates of application.

Simazine is often used in combination with other herbicides including paraquat, atrazine, and amitrole. Formulations are available as wettable powders, granulars, and liquids.

Simazine is also registered for use in controlling algae in ponds. Little of the simazine parent chemical is found as residues in food and feed crops.

The Health Effects Division (HED) of OPP has received new toxicity studies on simazine following the Data Call-In Notice of the first Registration Standard of 1984. Reviews of these studies indicate that increased incidences of mammary tumors in female rats are associated with exposure to simazine in the diet.

Toxicology Branch I (IRS) of HED submits the data reviews for evaluation and asks for Peer Review determination of the appropriate oncogenic classification of the compound.

Acute Toxicity

Simazine technical has a low acute toxicity with the rat oral LD_{50} > 5 g/kg (Toxicity Category IV) and another rabbit dermal LD_{50} > 2.0 g/kg in "limit tests."

Inhalation data in rats show that at 1.71 mg/L (the sustained maximum generated) minimal toxicity signs were evident following a 4-hour exposure (Toxicity Category IV).

Simazine is only very slightly irritating to the skin of rabbits after a 4-hour exposure (Toxicity Category IV), and is not a dermal sensitizer to guinea pigs.

Developmental Toxicity

A rat teratology study using dosages by gavage of 0, 30, 300, or 600 mg/kg exhibited maternotoxicity and fetotoxicity at 300 mg/kg and above. No malformations were reported; toxicity to the fetuses was characterized by incomplete ossification. The NOEL for the study was 30 mg/kg. Toxicity was also reported in a rabbit teratology study as nonossification of bones and reduced fetal weights; the NOEL was 75 mg/kg. Maternal toxicity was reported at 75 mg/kg. Terata formation was not evident in the study.

Subchronic (90-Day) Studies

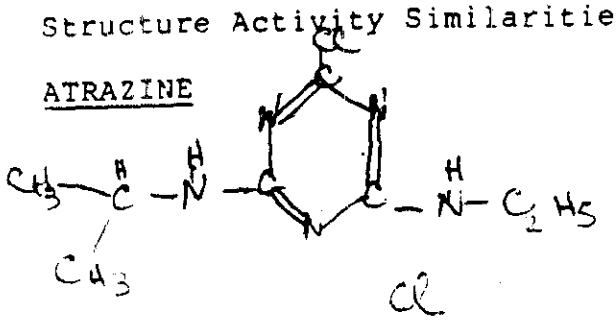
Rodent - Technical grade simazine was fed to rats in groups of 10/sex in a powdered feed mixture at 0, 200, 2000, or 4000 ppm. Reductions in feed intake and mean body weights occurred at 2000 ppm and above. A NOEL for males based on a reduction in red blood cells (RBC) counts was less than 200 ppm (LDT). Cholesterol and inorganic phosphate levels were elevated in both sexes. Renal stones were increased at 200 ppm and above when compared to controls. The LEL was less than 200 ppm (LDT) (Attachment 1).

Nonrodent - Beagle dogs in groups of 4/sex were exposed to dietary mixtures of 0, 200, 2000, or 4000 ppm simazine for 13 weeks. Clinical chemistries and hematological determinations were made midway and at termination of the study. Body weights, food and water intake, and clinical observations were also recorded.

Results - Tremors were present from 9 weeks to termination at 4000 ppm. Body weights and food consumption were reduced at 2000 ppm and above in both sexes. Reduced erythrocyte counts occurred at high doses. A NOEL was based upon reduced albumin and increased globulin levels in males. The MTD was less than 2000 ppm in both sexes based on the reduced body weights and food consumption values (Attachment 2).

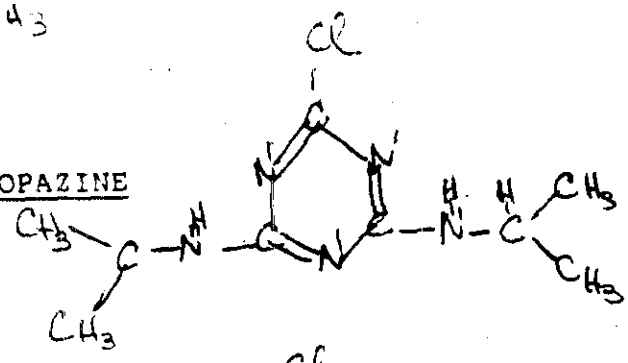
Structure Activity Similarities:

ATRAZINE



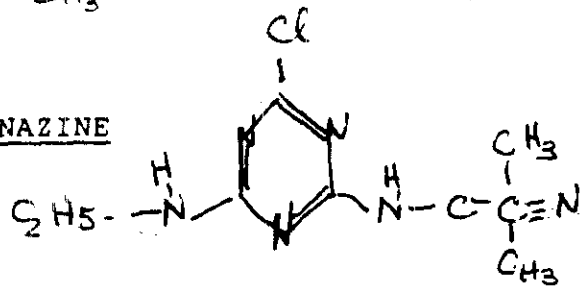
Animal Response
Increased female mammary gland tumors in the albino, rat. Peer reviewed as a "C Q*" oncogen.

PROPAZINE



Increased female CD-1 rat mammary gland tumors. Peer reviewed as a "C Q*" oncogen.

CYANAZINE



Not evaluated due to inadequate data.

Metabolism - Rats were fed 1.5 mg/kg ^{14}C ring-labeled simazine or metabolites obtained from fish fed simazine. The simazine-treated rats excreted 41 percent of the radioactivity in the feces and 49 percent in the urine. Animals fed the fish metabolites excreted 48 to 93 percent activity in the feces and 17 to 31 percent in the urine. Very small amounts of ^{14}C activity remained in the rats after 96 hours (Attachment 3).

A further study in rats indicated that simazine remained attached to RBC preferentially following oral dosing of the animals (Attachment 4).

Mutagenicity (Attachments 5, 6, 7)

Recent studies using simazine in mutagenicity evaluations have been received and provide information that an Ames assay using five doses ranging from 10 to 250 micrograms (ug)/plate was assayed at the maximum test doses possible with no evidence of mutagenic effect. Strains TA1535, TA100, TA1538, TA98, and TA1537 were used with and without S9 microsomal activation.

Structural chromosomal aberration tests were completed using human lymphocytes in vitro. The studies used both activation and nonactivation with 59 materials at concentrations of 6.25, 12.5, 25, 50, and 100 ug/mL. However, the studies were considered unacceptable because they could have been run at higher levels and posttreatment harvest time was extended beyond an optimal time period.

Unscheduled DNA repair in primary rat hepatocytes was evaluated but used too short incubation periods and presented insufficient information on dosage selection to be usable in the assay. The study was unacceptable to properly evaluate UDS.

Chronic Toxicity

Mice - Simazine was fed in the diet at levels of 0, 40, 1000, or 4000 ppm to groups of CD-1 mice containing 60 animals/sex for oncogenicity evaluation, and additional groups of 10/sex/dose for interim sacrifices at 26 and 52 weeks. Animals were observed daily and failed to show effects related to treatment at any dosage. Body weight gains were reduced at 1000 ppm and above in both sexes. Hematologic (Hct, Hgb, and RBC) changes were noted in females at 1000 ppm and above. Females appeared to be more sensitive to ingestion of simazine since most hematological effects in males were noted at 4000 ppm. Decreased organ to body weight ratios and absolute organ weights generally paralleled the lowered body weights observed in the test animals.

Neoplastic lesions were not increased significantly over values reported in control animals.

The study showed a NOEL of 40 ppm with no evidence for oncogenic potential (Attachment 8).

Rats - A chronic feeding study in Sprague-Dawley rats was used to examine simazine for oncogenic potential. Fifty rats/sex/dose were exposed to 0, 10, 100, or 1000 ppm of simazine in the diet and examined after 2 years for oncogenicity. Additional groups (30 to 40/sex/dose) were treated to determine toxicity endpoints (Attachment 9).

Survival - Male rats at the highest dose survived better than controls but females had a 20 percent survival rate compared to 34 percent in the controls.

Non-neoplastic Toxicity

Reduced body weight gains were seen in mid- and high-dose animals of both sexes. Food consumption was reduced signifi- significantly at 1000 ppm in both sexes but only occasionally at 100 ppm. Hematological parameters (Hgc, RBC, Hct) were variously depressed throughout the study at the mid- and high-dose levels in females. Clinical chemistry determinations show that glucose levels were lower at the mid and high doses in females when compared to controls. Other chemistry parameter changes were either not biologically significant or were not discernible as treatment-related.

Organ weight to body weight or brain weight changes were quite severe in the kidneys and livers of females on diets of 1000 ppm simazine. However, the great loss in body weights confounded the results. More likely, the absolute liver weights or percent of the brain weight would represent real changes in the organ weights from treatment. Therefore, the LEL for these effects was considered to be 100 ppm in females.

Neoplastic Changes (Excerpted from the TB review, Attachment 9)

Table 7. Summary of Histopathological Lesions - Male Rats

Histopathological Observation[1/]	Dose (ppm) 0	10	100	1000
Neoplastic Lesions				
Adrenal - Cortical adenoma	0/69[2/]	0/70	1/69	2/69
Kidney - Adenoma	0/70	0/70	0/70	1/70
- Carcinoma (primary)	0/70	0/70	0/70	2/70
Liver - Hepatocellular adenoma	1/70	1/70	1/70	3/70
- Hepatocarcinoma	0/70	2/70	4/70	3/70
- Combined adenoma and/or carcinoma	1/70	3/70	4/70	6/70
Thyroid - C-Cell adenoma	2/70	5/69	5/69	6/70
- C-cell carcinoma	2/70	1/69	1/69	3/70
- Combined adenoma and/or carcinoma	4/70	6/69	6/69	9/70

1/Main study only (interim sacrifice and recovery groups not included).
2/Number of rats with specified observation/total number of tissues examined.

HISTORICAL CONTROL TUMORINCIDENCE DATA
NUMBER OF TUMOR-BEARING ANIMALS - SPRAGUE-DAWLEY RATS

Submitted by Ciba-Geigy

	83	JAN 83	NOV 83	84	85	85	85
COMPOUND	A	B	C	D	E	F	G
SITE: NEOPLASM	NUMBER OF NEOPLASMS						
MAMMARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	6	6	8	2	5	3	2
FIBROADENOMA	18	16	26	21	12	23	22
ADENOMA/FIBROADENOMA (COMBINED)	22	18	30	22	15	25	23
ADENOCARCINOMA	7	4	5	11	9	15	14
ALL MAMMARY TUMORS (COMBINED)	25	22	34	30	20	34	32
PITUITARY GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(63)	(60)	(69)	(69)	(60)	(70)	(70)
ADENOMA	52	49	55	59	49	62	62
CARCINOMA	0	2	2	2	6	2	1
ADENOMA AND CARCINOMA (COMBINED)	52	51	57	61	55	64	63
KIDNEY (MALES AND FEMALES):							
NUMBER OF SITES EXAMINED	(65/65)	(60/59)	(70/70)	(70/70)	(60/60)	(70/70)	(70/70)
	M F	M F	M F	M F	M F	M F	M F
ADENOMA	0 0	0 0	2 0	1 0	0 0	0 0	0 0
CARCINOMA	0 0	0 0	0 0	1 0	0 0	0 0	0 0
ADENOMA AND CARCINOMA (COMBINED)	0 0	0 0	2 0	2 0	0 0	0 0	0 0
ADRENAL GLAND (FEMALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	1	3	4	2	3	2	8
LIVER (MALES):							
NUMBER OF SITES EXAMINED	(65)	(60)	(70)	(70)	(60)	(70)	(70)
ADENOMA	0	2	0	2	10	4	1
CARCINOMA	0	1	1	6	2	1	0

Table 6. Simazine, Sprague-Dawley Rat Study--Female Pituitary Gland Tumor Rates+, Fatal Tumor Analysis and Generalized K/W Test Results (extracted from T.B. review, Attachment 9)

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	73/89 (82.0)	57/80 (71.2)	63/77[a] (81.8)	61/79 (77.2)
	p = 0.0033**	p = 0.9944	p = 0.0206*	p = 0.0030**
Carcinoma	1/73 (1.4)	3/61 (4.9)	0/52 (0.0)	6/53[b] (11.3)
	p = 0.0010**	p = 0.2351	p = 0.4545	p = 0.0153*
Adenoma/ Carcinoma	74/89 (83.1)	60/80 (75.0)	63/77 (81.8)	67/79 (84.8)
	p = 0.0005**	p = 0.8351	p = 0.0251*	p = 0.0005**

+Number of tumor-bearing animals/number of animals at risk (excluding animals that died before the first tumor or animals not examined).
() = Percent
[a]First adenoma observed at 35 weeks in dose 100 ppm.
[b]First carcinoma observed at 72 weeks in dose 1000 ppm.

Note: Significance of trend donated at control. Significance of pairwise comparison with control denoted at dose level.
*Denotes $p < 0.05$.
**Denotes $p < 0.01$.

Table 8. SINAZINE SPRAGUE-DAWLEY RAT Study-- Female Mammary Gland Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p= 0.0489	p= 0.302	p= 0.177	p= 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p< 0.0001**	p= 0.4740	p= 0.0392*	p< 0.0001**
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (78)
	p< 0.0001**	p= 0.4064	p= 0.2229	p< 0.0001**

a First Adenoma observed at 48 weeks in dose 10 ppm and the first Fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.

b First carcinoma observed at 48 weeks in dose 100 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Due to the presence of mortality differences in both sexes of rats, the Peto prevalence test was used for incidental tumor rates to test for increasing incidence with increasing dose levels and for pair-wise differences between controls and treated rats. If the Peto prevalence method reduces to too few intervals then the Cochran-Armitage method is used to test for trends and the Fisher's exact test to test for pair-wise differences. If the tumors are considered fatal, the Thomas, Breslow, and Gart procedure is used to analyze for trends and pair-wise differences.

In the female rats, M. Copley suggested that the mammary gland adenomas and fibroadenomas be analyzed together as benign tumors, since about 50% of the rats with fibroadenomas also had carcinomas. There were no significant pair-wise comparisons or a trend noted. There was a significant dose-related trend for mammary gland carcinomas and for combined mammary gland adenomas/fibroadenomas and carcinomas ($p < 0.0001$). The incidence of mammary gland carcinomas in the 100 ppm and 1000 ppm dose groups were significantly increased ($p = 0.0392$ and $p < 0.0001$, respectively) compared to the controls. The incidence of combined mammary gland adenomas/fibroadenomas and carcinomas in the 1000 ppm dose group was significantly increased ($p < 0.0001$) compared to the controls (Table 8).

Weight-of-the-Evidence

Data on simazine, although sparse, do not indicate a strong mutagenic potential. Chronic data in rat and mice studies indicate that simazine affects body weight gains and hematological parameters in the two species. The rat showed a NOEL of 0.5 mg/kg; the mouse exhibited a NOEL of approximately 6 mg/kg.

The mouse was negative for oncogenic effects associated with exposure to simazine at up to 4000 ppm in the diet.

The effects of simazine on the mammary glands of the female rat indicate increased oncogenic potential in that sex and species. There was also a significant increase in pituitary tumors. The mechanism of tumorigenicity was not discernible from the data submitted.

The male rat data showed an increase in kidney tumors as well as liver tumors.

In summary, one species, the rat, exhibited increased incidences of female mammary tumors and pituitary tumors. The male rat exhibited a dose-related trend for increased kidney tumors and significant numbers (Fisher's Exact test) of liver tumors.

Attachments

Attachment 10 Tab 1 Index

OCT 28, 88

EPA: 68-D8-0565
DYNAMAC No. 1-16
October 18, 1988

SIMAZINE - Qualitative Risk Assessment from a Rat Two Year Oral Chronic Toxicity and Oncogenicity Study

Caswell No. 740

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Date: Oct. 20, 1988

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Date: 10-21-88

SUMMARY:

Simazine technical was fed to male and female Sprague-Dawley rats at doses of 0, 10, 100, or 1000 ppm in a 104 week chronic toxicity/oncogenicity study.

For female rats, there was a statistically significant increase in mortality with increasing doses of Simazine and mortality was significantly increased in both the 100 and 1000 ppm dose groups compared to the controls.

The incidence of mammary gland carcinomas and combined adenomas and carcinomas had a significant dose-related trend. The incidence of mammary gland carcinomas was significantly increased compared to the controls at the 100 and the 1000 ppm groups; the combined adenomas and carcinomas was significantly increased compared to the controls for the 1000 ppm group.

The pituitary gland tumors were considered fatal (reference page 1460 of the Ciba-Geigy report, attached), all three tumor groups (adenomas, carcinomas, and combined adenomas and carcinomas) showed significant dose-related trends. The incidence of pituitary adenomas and combined tumors was significantly increased compared to controls at the 100 and 1000 ppm groups; the incidence of carcinomas was significant at the 100 ppm group only.

There was a significant dose-related trend for kidney tubule adenomas.

For male rats, there was a statistically significant decrease in mortality with increasing doses of Simazine and mortality was significantly decreased in the 1000 ppm group compared to the controls.

There were no significant dose-related trends for liver adenomas, carcinomas, and combined adenomas and carcinomas. The incidence of liver carcinomas in the 100 ppm group was significantly increased compared to the controls. The incidence of combined liver adenomas and carcinomas was significantly increased compared to the controls in the 1000 ppm group. There were no significant dose-related trends or pair-wise differences for thyroid C-cell adenomas, carcinomas, and combined adenomas and carcinomas.

There was a significant dose-related trend for kidney tubule carcinomas and combined adenomas and carcinomas. There were no significant pair-wise differences for any of the kidney tubule tumors.

BACKGROUND:

Simazine technical was fed to male and female Sprague-Dawley rats at doses of 0, 10, 100, or 1000 ppm in a 104 week chronic toxicity/carcinogenicity study. Approximately 10 animals in each sex were sacrificed after 52 weeks of continuous dosing in each dose group. Only 9 animals were sacrificed in the male 10 ppm dose group and in the female 100 and 1000 ppm dose groups. This was due to deaths on study which occurred before the scheduled sacrificed since the animals to be sacrificed were selected prior to the beginning of the study. Also ten animals from the 1000 ppm group are not included in this analysis. These animals were dosed for 52 weeks and then maintained for 52 additional weeks on an untreated (control) diet. They were designated as a recovery group. A supplementary table of the results from these animals and their assigned controls was prepared (attachment 1). There were only 2 kidney tumors in the males, one adenoma in the control group and one carcinoma in the 1000 ppm group. In the females, there were 4 mammary gland adenomas in the controls and 2 in the 1000 ppm group. There was 1 mammary gland carcinoma in the controls and 4 in the 1000 ppm group. There were no pituitary gland carcinomas in either group but there were 9 denomas in both groups.

The study was conducted by Ciba-Geigy Corporation, Pharmaceutics Division, Summit, NJ for the Ciba-Geigy Corporation. The TOX Chemical No. is 740, the MRID No. is 406144-05, and the Study No. is 2-011-09. Data was extracted from a final report dated April 12, 1988. Test animals were assigned randomly to the following dose groups:

Table 1. Experimental Design for Rat Chronic/Carcinogenicity Study

Dose (ppm)	Phase	Total Number		Time of Sacrifice 52 Weeks		Least Number of Dose Weeks
		Male	Female	Male	Female	
Control	Chronic c	10	10	10	10	52
		10	10			52 + 52-wk recovery
		20	20			104
	Carcinogenicity	50	50			104
10	Chronic c	10	10	10a	10	52
		20	20			104
	Carcinogenicity	50	50			104
100	Chronic c	10	10	10	10a	52
		20	20			104
	Carcinogenicity	50	50			104
1000	Chronic c	10	10	10	10a	52
		10b	10b			52 + 52-wk recovery
		20	20			104
	Carcinogenicity	50	50			104

a Only 9 animals were actually sacrificed in these dose groups.
b These 10 animals were excluded from analysis.
c The chronic animals were also used for hematology, biochemistry, and urinalysis.

SURVIVAL ANALYSIS:

In female rats, a statistically significant increasing trend in mortality was observed with increasing doses of Simazine ($p = 0.0036$). Mortality was significantly increased in the 100 ppm and the 1000 ppm dose group compared to the controls ($p = 0.0058$ and $p = 0.0006$ respectively). (Table 2).

In male rats, a statistically significant decreasing trend in mortality was observed with increasing doses of Simazine ($p = 0.0016$). Mortality was significantly decreased in the 1000 ppm dose group compared to the controls ($p = 0.0077$) (Table 3) .

Tests for mortality were made using the Thomas, Breslow, and Gart procedure. The earlier deaths occurred in the mid and high dose groups and the K/W test gives more weight to earlier deaths. Hence, all mortality test reported are the generalized K/W test.

TABLE 2. SINAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Mortality Rates* and Generalized K/W Test Results

DOSE(PPM)	WEEKS 1-26	27-52	52a	53-78	79-106a	TOTAL
0.000	0/90 (0)	1/90 (1)	10/10	13/79 (16)	39/66 (59)	53/80** (66)
10.000	0/80 (0)	2/80 (2)	10/10	18/68 (26)	27/50 (54)	47/70 (67)
100.000	1/80 (1)	8/79 (10)	9/9	18/62 (29)	26/44 (59)	53/71** (75)
1000.000	0/80 (0)	5/80 (6)	9/9	21/66 (32)	31/45 (69)	57/71** (80)

TABLE 3. SINAZINE, SPRAGUE-DAWLEY RAT Study--MALE Mortality Rates* and Generalized K/W Test Results

DOSE(PPM)	WEEKS 1-26	27-52	52a	53-78	79-106a	TOTAL
0.000	0/90 (0)	2/90 (2)	10/10	12/78 (15)	34/66 (52)	48/80** (60)
10.000	0/80 (0)	1/80 (1)	9/9	8/70 (11)	38/62 (61)	47/71 (66)
100.000	0/80 (0)	0/80 (0)	10/10	6/70 (9)	33/64 (52)	39/70 (56)
1000.000	0/80 (0)	0/80 (0)	10/10	6/70 (9)	22/64 (34)	28/70** (40)

* Number of animals that died during the interval/Number of animals alive at the beginning of the interval.

( ) Per cent

a Interim sacrifice was conducted at 52 weeks. Final sacrifice occurred at week 106.

Note: Time intervals were selected for display purposes only. Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

TUMOR ANALYSIS:

Due to the presence of mortality differences in both sexes of rats, the Peto prevalence test was used for incidental tumor rates to test for increasing incidence with increasing dose levels and for pair-wise differences between controls and treated rats. If the Peto prevalence method reduces to too few intervals then the Cochran-Armitage method is used to test for trends and the Fisher's exact test to test for pair-wise differences. If the tumors are considered fatal, the Thomas, Breslow, and Gart procedure is used to analyze for trends and pair-wise differences.

In the female rats, M. Copley suggested that the mammary gland adenomas and fibroadenomas be analyzed together as benign tumors, since about 50% of the rats with fibroadenomas also had carcinomas. There were no significant pair-wise comparisons or a trend noted. There was a significant dose-related trend for mammary gland carcinomas and for combined mammary gland adenomas/fibroadenomas and carcinomas ($p < 0.0001$). The incidence of mammary gland carcinomas in the 100 ppm and 1000 ppm dose groups were significantly increased ($p = 0.0392$ and $p < 0.0001$, respectively) compared to the controls. The incidence of combined mammary gland adenomas/fibroadenomas and carcinomas in the 1000 ppm dose group was significantly increased ($p < 0.0001$) compared to the controls (Table 4).

There was a significant dose-related trend for kidney tubule adenomas ($p = 0.0042$) by the Cochran-Armitage trend test (Table 5). The Cochran-Armitage trend test was used since the Peto prevalence procedure reduced to one interval. There were no significant pair-wise differences found using the Fisher's exact test for pair-wise differences.

A fatal tumor analysis was performed on female rat pituitary gland tumors (reference page 1460 of the Ciba-Geigy report, attached) and the generalized K/W analysis test results reported. There was a significant dose-related trend for pituitary gland adenomas only, carcinomas, and combined adenomas and carcinomas ($p = 0.0033$, $p = 0.0010$, and $p = 0.0005$ respectively) (Table 6). The incidence of pituitary gland adenomas in the 100 ppm and the 1000 ppm dose group was significantly increased ($p = 0.0206$ and $p = 0.0030$ respectively). The incidence of pituitary gland carcinomas was significantly different from the controls in the 1000 ppm dose group ($p = 0.0153$). The 100 ppm and 1000 ppm dose group of combined pituitary adenomas and carcinomas was significantly different from the controls ($p = 0.0251$ and $p = 0.0005$ respectively).

From an examination of the Kaplan-Meier survival curves (copies available), the pituitary adenoma/carcinoma lesions appear 4 to 15 weeks earlier in the 100 ppm and 1000 ppm dose

groups than they do in the 10 ppm dose or control groups. The incidence of the mid and high group remain higher than the other two groups until near the end of the study.

In the male rats, there were no dose-related trends for liver adenomas, carcinomas, or combined liver adenomas and carcinomas by the Cochran-Armitage trend test (Table 7). The incidence of liver carcinomas in the 100 ppm group was significantly increased ($p = 0.0494$) compared to the controls by the Fisher exact test. The incidence of combined liver adenomas and carcinomas in the 1000 ppm group was significantly increased ($p = 0.0449$) compared to the controls. The Cochran-Armitage trend test and the Fisher's exact test were used because only one interval was calculated using the Peto prevalence test. For the liver carcinomas, animals that died before 52 weeks were excluded from analysis, although the first carcinoma appears at week 99. It was assumed that 52 weeks was an adequate time period for liver tumors to appear.

There were no significant pair-wise differences or dose-related trends for thyroid C-cell adenomas, carcinomas or combined thyroid C-cell adenomas and carcinomas (Table 8).

There was a significant dose-related trend for kidney tubule carcinomas and combined kidney tubule adenomas and carcinomas ($p = 0.0332$ and $p = 0.0056$, respectively) (Table 9). There were no significant pair-wise differences between treated groups and the controls for kidney adenomas, carcinomas or combined adenomas and carcinomas. Analysis of kidney tubule adenoma was done with the Cochran-Armitage trend test and Fisher's exact test since the Peto prevalence procedure resulted in only one interval.

Table 4. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Mammary Gland Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p= 0.0689	p= 0.302	p= 0.177	p= 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p< 0.0001**	p= 0.6740	p= 0.0392*	p< 0.0001**
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (78)
	p< 0.0001**	p= 0.4064	p= 0.2229	p< 0.0001**

a First Adenoma observed at 48 weeks in dose 10 ppm and the first Fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.
b First carcinoma observed at 48 weeks in dose 100 ppm.

Table 5. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Female Kidney Tubule Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	0/74 (0.0)	0/62 (0.0)	0/54 (0.0)	2/55c (3.6)
	p= 0.0042**	p= 1.0000	p= 1.0000	p= 0.1799

c First Adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.
* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animal not examined).
( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. SIMAZINE, SPRAGUE-DAWLEY RAT Study--FEMALE Pituitary Gland Tumor Rates*, Fatal Tumor Analysis and Generalized K/W Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	73/89	57/80	63/77 a	61/79
	(82.0)	(71.2)	(81.8)	(77.2)
	p= 0.0033**	p= 0.9944	p= 0.0206*	p= 0.0030**
Carcinoma	1/73	3/61	0/52	6/53 b
	(1.4)	(4.9)	(0.0)	(11.3)
	p= 0.0010**	p= 0.2351	p= 0.4545	p= 0.0153*
Adenoma Carcinoma	74/89	60/80	63/77	67/79
	(83.1)	(75.0)	(81.8)	(84.8)
	p= 0.0005**	p= 0.8351	p= 0.0251*	p=0.0005**

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the first tumor or animals not examined).

( ) Per cent

a First Adenoma observed at 35 weeks in dose 100 ppm.

b First Carcinoma observed at 72 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p > 0.01$

Table 7. SIMAZINE SPRAGUE-DAWLEY RAT Study·· Male Liver Tumor Rates• and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	1/88	2/79a	0/80	3/80
	(1.1)	(2.5)	(0.0)	(3.8)
	p= 0.0824	p= 0.4594	p= 0.5238	p= 0.2752
Carcinoma	0/88	2/79	4/80b	3/80
	(0.0)	(2.5)	(5.0)	(3.8)
	p= 0.2169	p= 0.2223	p= 0.0496*	p= 0.1058
Adenoma				
Carcinoma	1/88	4/79	4/80	6/80
	(1.1)	(5.1)	(5.0)	(7.5)
	p= 0.0443	p= 0.1519	p= 0.1554	p= 0.0449*

a First Adenoma observed at 52 weeks in dose 10 ppm.

b First Carcinoma observed at 99 weeks in dose 100 ppm.

• Number of tumor bearing animals/Number of animals at risk (excluding animals that died before 52 weeks or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 8. SIMAZINE SPRAGUE-DAWLEY RAT Study·· Male Thyroid C-Cell Tumor Rates• and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	2/52	7/52a	5/51	6/58
	(4)	(13)	(10)	(10)
	p= 0.3355	p= 0.0606	p= 0.1082	p= 0.0870
Carcinoma	2/34	1/31	1/36	3/45b
	(6)	(3)	(3)	(7)
	p= 0.1762	p= 0.1082	p= 0.2881	p= 0.4183
Adenoma				
Carcinoma	4/52	8/52	6/51	9/58
	(8)	(15)	(12)	(16)
	p= 0.1924	p= 0.1965	p= 0.2261	p= 0.1505

a First Adenoma observed at 89 weeks in dose 10 ppm.

b First Carcinoma observed at 102 weeks in dose 1000 ppm.

• Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

Table 9. SIMAZINE SPRAGUE-DAWLEY RAT Study-- Male Kidney Tubule Tumor Rates* and Peto Prevalence Test Results

DOSE(PPM)	0.000	10.000	100.000	1000.000
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57a (2)
	p= 0.0563	p= 1.0000	p= 1.0000	p= 0.5278
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65b (3)
	p= 0.0332*	p= 0.1660	p= 0.1821	p= 0.2091
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)
	p= 0.0056**	p= 0.1610	p= 0.1721	p= 0.1087

a First Adenoma observed at 92 weeks in dose 1000 ppm.

b First Carcinoma observed at 78 weeks in dose 1000 ppm

c The p values for Adenomas were calculated using the Cochran-Armitage Trend Test and Fisher's Exact Test, since the Peto Prevalence method collapsed to one interval.

* Number of tumor bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).

( ) Per cent

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

REFERENCES:

Thomas, D.G., N. Breslow, and J.J. Gart, Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381, 1977.

Cochran, W.G. Some Methods for Strengthening the Common x2 Test. Biometrics 10, 417-451, 1954.

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Peto, R., M Pike, P Day, P Gray, S Parish, J Peto, S Richard, and J Wahrendorf Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic effects in Long-term Animal Experiments. Monograph on the Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal. International Agency on Research on Cancer Monograph - Supplement 2, 311-426, 1980.

ATTACHMENT 1. SIMAZINE Female Rat Tumor Rates in rats fed 1000 ppm for 52 weeks and then allowed a 52-week recovery period compared to their matching control groups.

Tumor	Dose(ppm)	0	1000	0	1000
Mammary Gland:		FEMALES		MALES	
Adenoma and/or Fibroadenoma		4/10	2/10		
Carcinoma		1/10	4/10		
Adenoma/Fibroadenoma/ Carcinoma Combined		5/10	6/10		
Pituitary:					
Adenoma only		9/10	9/10		
Carcinoma		0/10	0/10		
Adenoma and/or Carcinoma		9/10	9/10		
Kidney Tubules:					
Adenomas		0/10	0/10	0/10	1/10
Carcinoma				1/10	0/10
Adenoma and/or Carcinoma				1/10	1/10
Liver:					
Adenoma only				0/10	0/10
Carcinoma				0/10	0/10
Adenoma and/or Carcinoma				0/10	0/10
Thyroid C-Cell:					
Adenomas				0/10	0/10
Carcinoma				0/10	0/10
Adenoma and/or Carcinoma				0/10	0/10

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Cyanazine (188C), Atrazine (63) and Simazine (740) Quantitative Risk Assessment Comparisons on Malignant Mammary Gland Tumors only in Rats. Revised Comparisons as of July, 1991.

From: Bernice Fisher, Biostatistician
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

Bernice Fisher 7/8/91

To: Karl Baetcke, Ph.D., Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Kerry L. Dearfield, Ph.D., Acting Section Head
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)
and
Reto Engler, Ph.D., Chief
Scientific Analysis & Coordination Branch
Health Effects Division (H7509C)

Kerry L Dearfield 7.8.91

HED's previous estimate of cyanazine's Q_1^* of 8.8×10^{-1} was based upon malignant mammary gland tumors including fibrosarcomas. For comparative purposes with atrazine and simazine, malignant tumors including adenocarcinomas, carcinomas and carcinosarcomas only are used in the estimation of the unit risk, Q_1^*.

Animals with fibrosarcomas in the cyanazine study are excluded from the group for the estimate of Q_1^*. The reason for this exclusion is due to advice given by Dr. Brennecke (HED's consultant in pathology) that fibrosarcomas do not originate from epithelial cell tissues as do the carcinomas. The carcinosarcomas, which originate from both the epithelial and mesenchymal cell tissues, found in both the atrazine and cyanazine mammary gland malignant tumor data can be retained for the estimate of Q_1^*.

cc Kathy Pearce SRRD

Table on Estimated[+] Q_1^* $(mg/kg/day)^{-1}$ for Cyanazine, Atrazine and Simazine in Sprague-Dawley Female Rats

	Tumors in the Mammary Gland	Q_1^* $(mg/kg/day)^{-1}$ Rat	In Human Equiv.[++]
Cyanazine	Carcinosarcomas & Adenocarcinoma	1.59×10^{-1}(a)	8.4×10^{-1}(c)
Atrazine	Adenocarcinoma & Carcinosarcoma	1.72×10^{-2}(b)	9.2×10^{-2}(c)
Simazine	Carcinoma	2.25×10^{-2}(b)	1.2×10^{-1}(c)

[+] Based on results from Statox computer program
[++] Derived by the use of surface area correction - $(\text{Human Wt.}/\text{Rat Wt.})^{1/3}$

(a) Multi-Stage Model (Global86)
(b) Time-to-Tumor Multi-Stage Model (Weibull83)
(c) Cyanazine - This Q_1^* is the estimate to be used for Risk Characterization.
Atrazine - This Q_1^* is the estimate for comparative purposes only of the three chemical compounds and is not the one that is used for Risk Characterzation (actual estimate used is 2.2×10^{-1} based upon both benign and malignant mammary gland tumors).
Simazine - This Q_1^* is the estimate that has been and is still being used for Risk Characterization.

Watts and Duplicates

SUPPLEMENTAL INFORMATION

DATA EVALUATION RECORD

ATRAZINE/080803

STUDY TYPE: NON-GUIDELINE - EFFECT ON LUTEINIZING HORMONE SURGE - RAT
MRID 45622309

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-52

Primary Reviewer:
H. Tim Borges, Ph.D., D.A.B.T., MT(ASCP) Signature: _____
Date: _____

Secondary Reviewers:
Carol Forsyth, Ph.D., D.A.B.T. Signature: _____
Date: _____

Kowetha A. Davidson, Ph.D., D.A.B.T. Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Artensie Flowers, Ph.D., MPH **Signature:** _____
Science Information Mgt. Branch, Health Effects Division (7509C) **Date**_____
EPA Secondary Reviewer: Joycelyn Stewart, Ph.D. **Signature:** _____
Toxicology Branch, Health Effects Division (7509C) **Date**_____

DATA EVALUATION RECORD
TXR#: 0050773

STUDY TYPE: Luteinizing Hormone Surge - Rat
Non-Guideline

PC CODE: 080803 **DP BARCODE:**D281938
SUBMISSION NO.: S612886

TEST MATERIAL (PURITY): Atrazine (97.1%),
Simazine (98.3%)
DACT (96.8%)

SYNONYMS: Atrazine - Atrex; 6-chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine
Simazine - CDT; Simadex; 2-chloro-4,6-bis(ethylamino-1,3,5-triazine
DACT; Diaminochlorotriazine; 6-chloro-1,3,5-triazine-2,4-diamine

CITATION: Minnema, D.J. (2002). 52-Week toxicity study of simazine, atrazine, and DACT administered in the diet to female rats. Covance Laboratories Inc., 9200 Leesburg Pike, Vienna, VA. Laboratory Identification Number 6117-399. February 21, 2002. MRID 45622309. Unpublished

Sielken, R.L. (2002). Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine, or DACT for Six Months: Statistical Analysis of the LH Surge: Supplemental Analysis. Sielken and Associates Consulting, Inc., Bryan, TX. Laboratory Identification Number 6117-399. March 4, 2002. MRID 45629402. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: A study was done to evaluate the effects of atrazine (Lot No. SG8029BA10, purity 97.1%), simazine (Lot No. SG202028GB10, purity 98.3%), or diamino-chlorotriazine (DACT) (Lot No. GP-720301, purity 96.8%) treatment on the estrous cycle and luteinizing hormone (LH) surge in response to exogenously administered estrogen to female rats after ~ 6 months of treatment and on the female reproductive organs after 52 weeks of treatment (MRID 45622309). For six months, female Sprague-Dawley Crl:CD®(SD)IGS BR rats received atrazine at 1.8, 3.4, 4.9, and 29.1 mg/kg/day (25, 50, 70, and 400 ppm), simazine at 1.6, 3.2, 4.6, 26.8 mg/kg/day (23, 47, 66, or 374 ppm), and DACT at 1.2, 2.4, 3.4, and 19.7 mg/kg/day (17, 34, 48, or 270 ppm). During the overall study, high-dose animals received 28.2, 25.9, and 18.8 mg/kg/day of atrazine, simazine, and DACT, respectively.

High doses of both atrazine and simazine decreased total body weight by the second week of the study and remained ~85% of control through the remainder of the study. Total body weight gain of female rats in the high-dose treatment groups of atrazine and simazine were ~70% of control at weeks 29 and 52. Treatment with high doses of DACT and with lower doses of atrazine and simazine had relatively little impact of total body weight and body weight gain. In contrast, food consumption of high-dose atrazine and simazine treated rats was essentially unaffected by treatment, suggesting food efficiency was decreased in these two high-dose groups. Food consumption was also not affected by treatment for female rats in lower dose atrazine and simazine groups, or in any dose groups treated with DACT.

No significant effect of treatment with high doses of atrazine, simazine, or DACT were apparent in vaginal smears assessing percent days in diestrus or estrus, or percent days in diestrous or estrous blocks. Six months after treatment of female rats with equal molar doses of atrazine or simazine, no effect on LH_{max} , LH AUC and $Time_{max}$ was found. However, LH_{max} and LH AUC were decreased in female rats treated for 6 months with high doses of DACT. Time to LH_{max} was unaffected by treatment with DACT. Similarly, when the concentration of plasma LH was analyzed by observation time, it was found that high-dose DACT decreased LH surge.

No macroscopic or microscopic treatment-related effects were found in tissues of the reproductive tract of female rats and no increase in brain weight was found. The incidence of pituitary adenomas was slightly increased in all three high-dose test material groups relative to the controls. In addition, the incidence of mammary carcinoma was increased in the high-dose DACT treatment group rats. The significance of these increases is uncertain since they were generally within the background percent incidence found in female rats of this strain.

In summary, atrazine and simazine behaved similarly in this study (i.e., no effect on LH surge), while DACT suppressed the exogenously stimulated LH surge. However, confidence in this study is low. Based on historical control data, the induction of LH surge in control animals appears to be suboptimal. Rats exhibited an infection that may have impacted LH induction. In addition, estradiol data were not submitted to evaluate LH induction response. Also, in a separate study (MRID# 44152102), atrazine had a significant effect on LH surge (LOAEL = 3.65 mg/kg/day; NOAEL = 1.8 mg/kg/day). A definitive effect on LH surge could not be determined, given the deficiencies of the study.

Based on decreased body weight and body weight gain, the systemic LOAELs for female rats treated with atrazine or simazine are ~29 mg/kg bw/day for atrazine and ~26.4 mg/kg bw/day for simazine. The corresponding NOAEL is 4.9 mg/kg bw/day for atrazine and 4.6 mg/kg bw/day for simazine. The systemic LOAEL for DACT is > 19.3 mg/kg bw/day and the systematic NOAEL for DACT is ~19.3 mg/kg bw/day.

A definitive effect on LH surge could not be determined, given the deficiencies of the study.

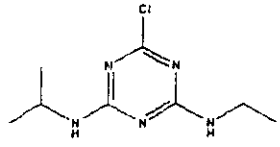
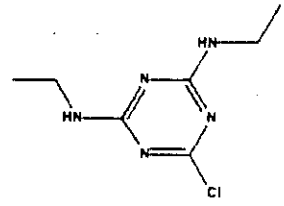
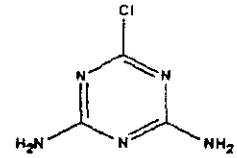
This LH surge study in the rat is **Unacceptable/Non-guideline** and does not satisfy the intent of investigating the effect of atrazine, simazine, and DACT on the LH surge of the female SD rats. A definitive effect on LH surge could not be determined, given the deficiencies of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:**

	Atrazine	Simazine	DACT
Description:	white powder	white powder	white powder
Lot/Batch #:	SG8029BA10	SG202028GB10	GP-720301
Purity:	97.1%	98.3%	96.8%
Compound Stability:	1 year	6 years	1 year
CAS # if TGAI:	1912-24-9	122-34-9	3397-62-4
Structure:			

2. **Vehicle and/or positive control:** Diet for study; sesame oil for replacement hormone

3. **Replacement Hormone:** Estradiol, purity 100%, Lot No. 77H37751

4. **Test animals:**

Species:	rat
Strain:	Sprague-Dawley Crl:CD®(SD)IGS BR
Sex:	female
Age/weight at study initiation:	young adult, 167-216 g
Source:	Charles River Laboratories, Raleigh, NC
Housing:	individually in hanging stainless steel cages
Diet:	Harlan Teklad Certified Rodent Diet, *ad libitum*
Water:	tap, *ad libitum*
Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air Changes:	10/hour
Light Cycle	14/10 light/dark
Acclimation period:	2 weeks

B. STUDY DESIGN:

1. **In life dates:** Begin - 08/24/1999; End - 09/06-07/2000

2. **Animal assignment**: Rats were assigned to the groups shown in Table 1 by using a computerized block randomization procedure based on body weight.

TABLE 1: Study design

Group	Test Material	Dietary Conc. (ppm)	Dose (mmoles/ kg feed)	# Females	
				Interim sacrifice (29 weeks)	Terminal sacrifice (52 weeks)
1	Control	-	-	32	50
2	Atrazine	25	0.116	16	-
3		50	0.232	16	-
4		70	0.325	16	-
5		400	1.854	16	20
6	Simazine	23	0.116	16	-
7		47	0.232	16	-
8		66	0.325	16	-
9		374	1.854	16	20
10	DACT	17	0.116	16	-
11		34	0.232	16	-
12		48	0.325	16	-
13		270	1.854	16	20

Data from p. 17, MRID 45622309

3. **Dose selection rationale:** Atrazine was used as the standard to set doses for simazine and DACT. The maximum tolerated dose for atrazine in the diet was 400 ppm based on the reduction of the luteinizing hormone (LH) surge occurring during normal cycling in the young female SD rat. The other dietary concentrations (25, 50, and 70 ppm) were chosen to demonstrate a no-observed effect level for LH surge, estrus cycle, and possibly tumorigenic effects. The dietary concentrations for simazine and DACT were chosen as molar equivalents.

4. **Diet preparation and analysis:** Diets were prepared weekly and adjusted to 100% chemical purity. The diets were prepared by adding the appropriate amount of test material to ~200 g diet and blending in a Waring blender until homogenous. The diets were mixed for ~1 minute/kg/10 kg (or ~10 minutes for batches <10 kg). Two samples/test material were taken from each mixed batch and stored at -20°C until analysis by HPLC or gas chromatography. Duplicate homogeneity analyses were done on the low- and high-dose diets for each test material. Diet stability analyses from the low- and high-dose levels of each test material were done to determine 10-day stability.

Results:

Homogeneity: The concentration of the three test materials were 97-103% of nominal in the top, middle, and bottom of all prepared diets.

Stability: All diets containing the test materials were stable for at least 10 days from preparation.

Concentration Analyses: All test material concentrations were within ± 10%.

5. **Statistics:** ANOVA was used for body weight, average body weight change, and mean food consumption. Statistical analyses for all treatment groups were compared against control, and in addition against the various test material groups. If data were heterogenous, it was rank transformed. If this failed to achieve homogeneity, ANOVA was still done.

   LH surge was assessed by: 1. Maximum increase in LH over baseline (LH_{max}); 2. time to peak LH surge (T_{max}); and area-under–curve for LH vs time (AUC).

   Multiple linear regression for each of the treatment materials was done on the scored daily vaginal smears for: 1. percentage of days in diestrus (% days D); 2. percentage of days in estrus (% days E); 3. percentage of days in diestrous blocks (% Days D-block [4 consecutive days in diestrous]); and 4. percentage of days in estrous blocks (% days E-blocks [2 consecutive days]). The control group was used as the 0 ppm group. Dose and week were used as the independent variables.

C. METHODS

1. **Cageside Observations:** The rats were observed twice daily for morbidity and moribundity. Body weights and food consumption were recorded weekly

2. **Estradiol Implantation:** The implants were prepared by slicing medical grade silastic silicon tubing into 20 mm lengths. Teflon beading (5 mm) was inserted into the tubing, made flush, and glued into place. Estradiol benzoate (4 mg/mL sesame oil) was inserted into the open end of the tubing followed by small cut sections of untrimmed Teflon beading that were cut flush with the end and sealed with adhesive.

3. **Determination of Cycling:** Daily vaginal smears following lavage with 0.9% saline were taken for 14 consecutive days every 4 weeks beginning on Day 1 through the end of the study. The lavage material was spotted on a glass slide, stained with toluidine blue and allowed to air dry. The smears were examined and scored as proestrus, estrus, or diestrus. The percent number of days spent in estrus was determined for each rat/group/treatment/time interval and the group mean and standard error of the mean determined. In addition, the percent of rats in each dose group that spent 7 or more days in estrous during each interval was calculated and the proportion of rats with normal estrous cycles determined. For the purposes of the study the estrous cycle was considered abnormal if 2 or more consecutive days were spent in either proestrous or estrus or 4 or more consecutive days were spent in diestrous.

4. **LH Surge:** Beginning on week 29, interim-sacrifice rats were ovariectomized. Because of the large number of rats involved, the ovariectomies were staggered over a period of 8 days. Six days after the ovariectomy, each rat was given an estradiol implant and 3 days later blood was collected to determine plasma LH. Blood was collected at 6 intervals during the day: 8, 11, and 13 hours after the lights were turned on, and 1, 3, and 5 hours after the lights were turned off. The plasma samples were stored at -70°C until time of analysis.

5. Necropsy: All rats found dead, killed *in extremis*, or sacrificed on schedule were subjected to gross pathological examination. After collection of the final blood sample, interim sacrifice

rats were weighed and sacrificed. After 52-weeks of treatment, the remaining surviving rats were weighed, sacrificed, and exsanguinated. The following tissues and organs were collected from all rats: mammary masses, mammary tissue, uterus, ovaries, vagina, brain (including hypothalamus), salivary glands, a section of skin with fur, and miscellaneous lesions.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed.

2. **Mortality:** No treatment-related effects on survival were observed.

B. BODY WEIGHT AND WEIGHT GAIN: Body weights for the three treatment regimens are shown in Table 2 and body weight gains are shown in Table 3. Mean body weight in the high dose group for all three test chemicals was lower than control throughout the study, with an apparent order of atrazine ≈ simazine >> DACT. High-dose atrazine and simazine consistently significantly decreased body weight throughout the study, while DACT significantly decreased body weight only up to week 44. These differences in body weight were present from the second week. Female rats receiving the mid high-dose of atrazine and simazine also had statistically significant decreases in body weight; however, the decreases were less than 6%. As would be expected, the body weight gain of female rats receiving the high dose of atrazine and simazine was also significantly decreased at 29 and 52 weeks. DACT significantly decreased body weight gain at week 29, but not week 52.

TABLE 2. Mean gram body weight (± SD) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]

Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1	193±8.8	192±10	193±10	191±8	192±8	190±9	191±11	193±10	192±8	193±8	192±7	192±9	192±9
2	214±11	212±10	212±12	210±14	204±8* (-5%)[b]	208±12* (-3%)	208±15	209±12	204±11* (-5%)	214±10	212±9	213±9	206±11* (-4%)
10	294±22	281±21* (-4%)	289±22	281±18* (-4%)	262±17* (-11%)	284±27	280±28* (-5%)	275±21* (-6%)	259±22* (-12%)	286±17	282±22* (-5%)	294±17	273±17* (-7%)
20	331±30	308±27* (-7%)	316±29	310±24* (-6%)	285±21* (-14%)	316±29	310±42* (-6%)	300±26* (-9%)	286±32* (-14%)	324±30	316±29	328±23	309±25* (-7%)
29	341±32	320±33* (-6%)	328±27	321±24* (-6%)	298±24* (-13%)	328±31	321±42* (-6%)	313±31* (-8%)	296±31* (-13%)	334±31	326±32	336±20	319±26* (-6%)
40	364±34	–	–	–	311±33* (-15%)	–	–	–	313±42* (-14%)	–	–	–	342±34* (-6%)
53	391±45	–	–	–	321±34* (-18%)	–	–	–	334±51* (-15%)	–	–	–	376±43 (-4%)

[a]Data from Table 4, pages 80-91 of MRID 45622309
[b]Results in parentheses are percent change relative to control
*$p \leq 0.05$

TABLE 3. Average body weight gain (g) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]													
Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1-29	148±27	126±23* (-15%)[b]	135±27	130±18	106±24*** (-28%)	136±24	125±30	114±29**	104±28*** (-30%)	137±37	124±36*	141±21	124±19*** (-16%)
1-52	199±43	–	–	–	129±32*** (-35%)	–	–	–	142±48*** (-29%)	–	–	–	185±40 (-7%)

[a]Data from Table 5, pages 92-105, MRID 45622309
[b]Results in parentheses are percent change relative control
*p≤0.05, **p≤0.01, ***p≤0.001 calculated by reviewer

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption**: In contrast to the significantly decreased body weights of rats treated with high doses of atrazine or simazine, food consumption of the high dose-group was only minimally decreased (≤12%) in these groups (Table 4). The decrease in food consumption of rats treated with high-doses of DACT was less (<7%). No significant treatment-related effects were found for the mid-high, mid, and low doses of atrazine, simazine, or DACT.

TABLE 4. Average food consumption (g) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]													
Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1	135±12	133±12	134±11	132±12	124±11* (-8%)	133±13	125±11* (-7%)	130±12*	122±9* (-10%)	134±14	134±12	136±12	127±11* (-6%)
2	145±15	141±12	137±13* (-6%)	136±14* (-6%)	133±15* (-8%)	138±11	132±13* (-9%)	136±11* (-6%)	128±9 (-12%)	140±13	140±15	140±15	135±10* (-7%)
10	136±14	131±13	131±17	134±13	131±16	130±13	127±13* (-7%)	129±10	124±14* (-9%)	128±15* (-6%)	127±13* (-7%)	139±15	136±10
20	144±17	137±19	142±17	137±12	133±15* (-8%)	136±15	132±18* (-8%)	134±12* (-7%)	132±18* (-8%)	145±18	138±20	147±16	145±14
29	148±21	144±24	139±26	142±17	137±17* (-7%)	139±17	135±18* (-9%)	137±18* (-7%)	134±17* (-9%)	140±25	138±21	149±23	149±16
40	146±17	–	–	–	137±18	–	–	–	138±20	–	–	–	147±14
52	153±16	–	–	–	144±25	–	–	–	144±16	–	–	–	152±14
1-52	7283	–	–	–	6928 (-5%)	–	–	–	6776 (-7%)	–	–	–	7181 (-1%)

[a]Data from Table 6, pages 106-118 of MRID 45622309
[b]Results in parentheses are percent of control
*p≤0.05

2. **Food efficiency**: Food efficiency was not reported in the study report; however, with the decreased body weights of high-dose atrazine and simazine rats and relatively normal food intakes for these groups, food efficiency would be decreased.

3. **Compound consumption**: Average compound consumption for the three test materials is shown in Table 5.

TABLE 5. Average compound consumption (mg/kg bw/day) of female rats treated with atrazine, simazine, or DACT for 29 or 53 weeks[a]

Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1 - 29	0	1.8	3.4	4.9	29.1	1.6	3.2	4.6	26.8	1.2	2.4	3.4	19.7
1 - 52	0	–	–	–	28.2	–	–	–	25.9	–	–	–	18.8

[a]Data from page 29 of MRID 45622309

D. ESTROUS CYCLE DETERMINATIONS:

Estrous cycles did not appear to be altered by any of the treatment compounds at any dose, as shown in Table 6 and Appendix 1. There were also no treatment-related effects on percent days in diestrus, percent days in diestrous blocks, or % days in estrous blocks (Appendix 1).

TABLE 6. Percent Days in Estrus [a]

Week	Control	Atrazine (ppm)				Simazine (ppm)				DACT (ppm)			
		25	50	70	400	23	47	66	374	17	34	48	270
1-2	22±5.1	22±3.6	21±5.8	21±6.0	23±4.6	21±4.1	26±4.4	23±4.1	23±5.8	22±4.9	23±5.4	22±4.3	22±4.1
5-6	23±4.3	23±3.2	23±3.2	23±5.3	24±8.7	22±3.4	24±7.3	23±4.1	22±4.8	24±4.4	21±5.5	23±3.9	26±9.4
9-10	24±5.0	24±3.6	33±27	33±25.1	28±17.1	22±4.4	26±4.3	28±11.2	28±12.3	24±4.4	24±3.4	22±4.4	25±14.7
13-14	31±18.2	34±25	34±27	33±26.5	34±26.8	34±26.7	25±4.5	28±14.8	38±28.0	29±17.7	22±1.8	28±17.9	32±23.1
17-18	37±30	40±32	43±34	37±26.6	46±35	41±36.3	40±29.3	44±28.6	42±31.9	40±31.7	27±20.6	36±29.8	41±31.7
21-22	47±34	49±33	48±36	41±30.4	53±34.4	50±36.0	53±33.0	51±31.7	51±35.4	50±35.6	34±27.5	46±36.8	50±32.2

[a]Data from Appendix 12, pages 1142-1147 of MRID 45622309
*$p \leq 0.05$

E. LUTEINIZING HORMONE:

As shown in Table 7, the evaluation of LH_{max}, AUC and T_{max} indicated that atrazine and simazine did not produce a treatment-related effect on LH surge, as compared to control. However, DACT at 270 ppm significantly decreased LH_{max} and AUC, but not T_{max}. Although not treatment-related, DACT at 34 ppm was found to increase LH_{max} and AUC. Similar treatment-related effects of DACT at the high dose (270 ppm) on LH surge are also found when analyzing the concentration of LH (ng/ml) at the observation time (Table 8). DACT significantly decreased the LH surge at 270 ppm, as indicated by the decrease in LH surge at 1400.

TABLE 7. Average LH_{max}, AUC, and T_{max} of female rats that received atrazine, simazine, or DACT for 29 weeks[a]

Dose (ppm)	No. Rats	LH_{max} (ng/mL)	AUC (ng-hr/mL)	T_{max} $(hr)^{-1}$
Control	26	1.872	5.370	14.385
Atrazine				
25	11	2.187	7.725	14.273
50	15	2.959	7.213	14.000
70	15	2.443	7.049	13.933
400	15	2.371	6.941	13.600
Simazine				
23	16	1.701	4.468	13.688
47	16	2.870	8.051	14.625
66	14	2.054	5.866	13.357
374	15	1.611	3.332	13.333
DACT				
17	13	3.361	8.866	13.846
34	12	3.618*	12.764*	13.083
48	14	2.496	6.355	14.643
270	16	0.750*	0.807*	13.375

Data from Table 10, p. 134, MRID 45622309
*$p \leq 0.05$

Table 8. Group Mean (±SD) Plasma LH (ng/ml)*

Dose group	Time				
	900	1200	1400	1600	1800
Control					
0	0.52±.46	0.93±.94	1.76±1.8	1.29±.95	0.84±1.16
Atrazine					
25	0.53±0.40	1.27±1.5	2.1±2.1	1.87±2.09	0.54±.34
50	0.63±.61	1.20±1.38	2.46±2.5	1.5±2.1	1.05±1.6
70	0.51±.53	1.14±.67	2.41±2.8	1.3±1.17	0.61±.53
400	0.56±.53	0.94±1.25	2.61±4.7	1.45±2.3	0.69±.67
Simazine					
23	0.73±.89	1.69±1.5	1.07±.88	1.30±1.3	0.95±.97
47	0.43±.51	0.85±.78	1.97±2.2	1.99±2.1	1.22±1.4
66	0.58±.48	1.24±.96	2.26±2.6	0.10±.91	0.58±.59
373	0.70±.80	1.62±2.6	0.94±1.5	1.07±1.2	0.50±.56
DACT					
17	0.41±.40	1.01±1.3	2.22±3.7	2.27±2.8	0.40±.26
34	0.77±.78	3.04±2.4	2.87±2.9	2.39±2.4	0.38±.28
48	0.71±.67	0.85±.79	2.08±1.3	2.37±2.2	0.65±.75
270	0.60±.65	0.79±.73	0.74±.56*	0.67±.52*	0.52±.51

* Data obtained from pages24-28, MRID 45269402???? (check this MRID)

F. SACRIFICE AND PATHOLOGY:

1. **Organ weight**: No treatment-related effects on brain weight, the only organ weighed at interim sacrifice, were found for female rats of the three treatment groups relative to control rats.

2. **Gross pathology**: No treatment-related effects were found in female rats of the three treatment groups at interim or final sacrifice, or in rats of unscheduled deaths.

3. **Microscopic pathology**: None of the three test materials induced treatment-related lesions in the reproductive organs examined. The incidence of pituitary adenomas in rats receiving the high-dose of the three test materials was increased relative to control rats (Table 9) and was significantly increased (as calculated by reviewer) for female rats in the DACT treatment group. However, the percent incidence was less than the spontaneous incidence of ~23% for rats of this species at 12-13 months of age. No increase in pituitary adenomas was found at lower doses of the three test materials. The incidence of mammary carcinoma was also

increased in female rats treated with a high dose of DACT, an incidence slightly greater than the maximum spontaneous increase of ~10% found in female rats of this species and age. No increase in mammary lesions were found in the lower-dose groups.

TABLE 9. Total incidence of selected neoplastic lesions found in female rats treated up to 52 weeks with high doses of atrazine, simazine, or DACT

Treatment (ppm)	No. Rats examined	Pituitary adenoma	Mammary Carcinoma	Mammary Fibroadenoma
Control (0)	82	7 (9%)*	4 (5%)	2 (2%)
Atrazine (400)	36	7 (19%)	0	0
Simazine (373)	36	5 (14%)	3 (8%)	0
DACT (270)	36	8 (22%)*	6 (17%)*	2 (6%)

Data from Table 19, p. 180-185, MRID 45622309
*Percent incidence
*p≤0.05 by Fishers exact test calculated by reviewer

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that female SD rats exposed to 270 ppm DACT in their feed for 29 weeks had decreases in LH surge (LH_{max} and AUC). A similar effect was not found in female rats treated with equal molar doses of atrazine or simazine. In addition, female rats treated with 270 ppm DACT had an increased incidence of pituitary adenomas, mammary carcinomas and mammary fibroadenomas. A slight increase in the incidence of pituitary adenomas were also found in female rats treated with high-doses of atrazine or simazine.

B. REVIEWER COMMENTS: In this study, female rats were treated with equal molar doses of atrazine, simazine, or DACT for a period of ~29 weeks and 52 weeks. High doses of both atrazine and simazine decreased total body weight by the second week of the study. The body weights of these two high-dose groups continued to decrease through week 10, where they remained ~85% of control through the remainder of the study. Total body weight gain of female rats in the high-dose treatment groups of atrazine and simazine were ~70% of control at weeks 29 and 52. Treatment with high doses of DACT and with lower doses of atrazine and simazine had relatively little impact on total body weight and body weight gain. In contrast, food consumption of high-dose atrazine and simazine treated rats was essentially unaffected by treatment, indicating food efficiency was decreased in these two high-dose groups. Food consumption was also not affected by treatment for female rats in lower dose atrazine and simazine groups, as well as all dose groups treated with DACT.

No significant effect of treatment with high doses of atrazine, simazine, or DACT were apparent in vaginal smears assessing percent days in diestrus or estrus, or percent days in diestrous or estrous blocks. A slight marginally significant effect noted for percent days in diestrous blocks of female rats treated with DACT was consistent with variability in response over time, particularly by rats treated with 34 ppm DACT over time. This response can be ruled out as treatment-related since the effect was not of the magnitude expected and showed no dose-related trend. Therefore, the reviewer considers treatment

with atrazine, simazine, and DACT as having no biologically significant effect on the estrous cycle.

Six months after treatment of female rats with equal molar doses of atrazine or simazine, no effect on the LH surge was found. However, LH_{max} and LH AUC were decreased in female rats treated for 6 months with the high dose of DACT. Time to LH_{max}, was not affected by treatment. However, confidence in this study is low. Based on historical control data, the induction of LH surge in control animals appears to be suboptimal. Rats exhibited an infection that may have impacted LH induction. In addition, estradiol data were not submitted to evaluate LH induction response. Also, in a separate study (MRID# 44152102), atrazine had a significant effect on LH surge (LOAEL = 3.65 mg/kg/day; NOAEL = 1.8 mg/kg/day). A definitive effect on LH surge could not be determined, given the deficiencies of the study.

No macroscopic or microscopic treatment-related effects were found in tissues of the reproductive tract of female rat and no increase in brain weights was found. However, the incidence of pituitary adenomas was increased in all three test material high-dose groups relative to the control group. In addition, the incidence of mammary carcinoma was increased in the high-dose DACT treatment group.

Based on decreased body weight and body weight gain, the systemic LOAELs for female rats treated with atrazine or simazine are ~29 mg/kg bw/day for atrazine and ~26.4 mg/kg bw/day for simazine. The corresponding NOAEL is 4.9 mg/kg bw/day for atrazine and 4.6 mg/kg bw/day for simazine. The systemic LOAEL for DACT is > 19.3 mg/kg bw/day and the systematic NOAEL for DACT is ~19.3 mg/kg bw/day.

A definitive effect on LH surge could not be determined, given the deficiencies of the study.

C. **STUDY DEFICIENCIES:** During weeks 21-23, many rats in all groups had an SDAV (Sialodacryoadenitis virus) infection. The effects of the infection included reduced food consumption, body weight loss, and ocular discharge. Because of the infection, the interim sacrifice was delayed from week 26 to week 29 to allow recovery from the infection. The study authors state that the impact of the infection on the LH surge is unknown. Infection may have impacted LH induction Based on historical control data, the induction of LH surge in control animals appears to be suboptimal. In addition, estradiol data were not submitted to evaluate LH induction response. Also, in a separate study (MRID# 44152102), atrazine had a significant effect on LH surge (LOAEL = 3.65 mg/kg/day; NOAEL = 1.8 mg/kg/day).

DATA FOR ENTRY INTO ISIS

Non-guideline - Rodent

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
080803	45622309	Non-guideline	rat	up to 52 weeks	oral	feed	0 - ~30	0, 1.8, 3.4, 4.9, ~29 atrazine 0, 1.6, 3.2, 4.6, ~26.4 simazine 1.2, 2.4, 3.4, ~19.3 DACT	4.9 atrazine 4.6 simazine 3.4 DACT	~29 atrazine ~26.4 simazine 19.7 DACT	Bdy wt Atra-zine & simazine Pituitary - DACT	None

APPENDIX 1

DATA FROM PAGES 46-51, MRID 45622309

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY

OPP/EPA Reviewer: Artensie R. Flowers, PhD, MPH **Signature:** _____
SIMB, Health Effects Division (7509C) **Date** _____
EPA Secondary Reviewer: Esther Rinde, PhD, D.A.B.T. **Signature:** _____
SIMB, Health Effects Division (7509C) **Date** _____

TXR#: 0050354

DATA EVALUATION RECORD

STUDY TYPE: Special Study (non-guideline). Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or Diaminochlorotriazine (DACT) via Oral Gavage for One Month.

PC CODE: 080807 (Simazine), 080803 (Atrazine) **DP BARCODE:** 277179
SUBMISSION NO.: S602042

TEST MATERIAL (PURITY): Simazine (100%), DACT (96.8%) , and Atrazine (97.1%).

SYNONYMS: G-27692 (Simazine); G-28273 (DACT); G-30027 (Atrazine)

CITATION: Minnema, D. Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or DACT via Oral Gavage for One Month. Covance Laboratories, Vienna, VA. Laboratory report number: 6117-398, March 21, 2001, MRID 45471002. Unpublished

SPONSOR: Syngenta Crop Protection, Inc., P.O. Box 18300 Greensboro, NC 27419

EXECUTIVE SUMMARY:

In a special study (MRID 45471002) on the effects of chlorotriazines on luteinizing hormone (LH) surge, simazine (100%, batch no. SG202028GB10), diaminochlorotriazine (DACT) (96.8%, batch no. GP720301) and atrazine (97.1% , batch no. SG8029BA10) were administered to 20 Sprague-Dawley Crl:CD BR female rats/dose/group by oral gavage at dose levels of 0, 2.5, 5, 40, 200 mg/kg bw/day (equivalent to 12.4, 24.8, 198.3, and 991.6 µmol//kg/day for simazine; for 17.2, 34.4, 274.9, 1374.6 µmol//kg/day for DACT; and 11.6, 23.2, 185.4, 927.2 µmol//kg/day for atrazine) once daily for at least 4 weeks. Vaginal smears were collected daily for the first 3 weeks of the study. On day 22, all animals were ovariectomized and six days later (day 28) surgically implanted (subcutaneously) with a capsule containing 4 mg estradiol/mL in sesame oil following the daily administration of the test material. On day 31, all animals received the daily dose, and subsequent blood serial samples were collected from each animal. At sacrifice, a number of tissues were collected (i.e. mammary, uterus, vagina and pituitary). Body weights were collected weekly, at the time of the estradiol implant, and on the day prior to sacrifice.

There were no compound-related deaths and no clinical signs of toxicity that could be attributed

to compound exposure. Administration of simazine, DACT, and atrazine was associated with mean body weight losses during the first week of the study at the 40 and 200 mg/kg/day dose levels for all three compounds. Over the duration of the 4-week study, significant decreases in body weight gains were noted at the 200 mg/kg/day dose level for all three compounds, and at the 40 mg/kg/day dose level for simazine and DACT. For most animals the peak LH concentration occurred at the 1800 hour interval. Results also showed that all three compounds had similar effects on diminishing the LH surge. All three compounds at the two highest doses, 40 and 200 mg/kg/day, significantly decreased adjusted peak LH surge. Analyses of pre-peak, peak, and post-peak LH concentrations, and comparison of responses between compounds (at the same dose levels) supported these results.

The LOAEL for systemic toxicity is 40 mg/kg/day for simazine, DACT, and atrazine, based on body weight effects. The NOAEL for all three compounds is 5 mg/kg/day.

The LOAEL for endocrine effects of atrazine, simazine, and DACT is 40 mg/kg/day, based on decreased adjusted peak LH concentrations. The NOAEL for endocrine effects is 5 mg/kg/day.

This special study on the endocrine effects of simazine, DACT, and atrazine is classified as an **acceptable-nonguideline** study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Atrazine

Description:	White powder
Lot/Batch #:	SG8029BA10
Purity:	97.1% a.i.
Compound Stability:	not provided
CAS # of TGAI:	122-34-9

2. Test Material: Simazine

Description:	White powder
Lot/Batch #:	SG202028 GB10
Purity:	100% a.i.
Compound Stability:	not provided
CAS # of TGAI:	1912-24-9

3. Test Material: Diaminochlorotriazine (DACT), metabolite

Description:	White powder
Lot/Batch #:	GP 720301
Purity:	96.8% a.i.
Compound Stability:	not provided
CAS # of TGAI:	N/A

4. Estradiol Beta-estradiol 3-benzoate

Description:	White powder
Lot/Batch #:	77H3775, E8515
Purity:	100% a.i.
Compound Stability:	not provided

5. Vehicle and/or positive control: control and vehicle for test material: 0.5% carboxymethylcellulose (Lot no. 87H0036); vehicle for replacement hormone (estradiol): sesame oil (Lot no. 37H0177)

4. Test animals:

Species:	female rat
Strain:	Sprague-Dawley Crl:CD® BR
Age at study initiation:	8 weeks old
Wt. at study initiation:	205-287 g
Source:	Charles River Laboratories, Inc Raleigh, NC
Housing:	individually in cages
Diet:	lab feed *ad libitum*

Water:	provided *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	10 or greater/hr
	Photoperiod:	14 hrs light/ 10 hrs dark
Acclimation period:	2 weeks	

B. PROCEDURES AND STUDY DESIGN

1. **Administration of test material and study schedule:** Animals were given the appropriate dosing formulation via oral gavage once daily for at least 4 weeks; dosing was performed at approximately 0630 (+/- 30 minutes) each day. Individual dose volume was based on the most recently recorded body weight and the dose factor of 10 ml/kg./day.

In the study timetable below (Table 1), Day 1 was the first day of dosing with the appropriate test material. Vaginal smears were collected daily for the first 3 weeks of the study. On day 22, all animals were ovariectomized. Following the daily dose on day 28, all animals were surgically implanted (subcutaneously) with a capsule containing 4 mg estradiol/mL in sesame oil. On day 31, all animals received the daily dose and subsequent blood serial samples were collected from each animal. Body weights were collected weekly, at the time of the estradiol implant, and on the day prior to sacrifice. At sacrifice, the mammary, uterus, vagina and pituitary tissues were collected.

TABLE 1. Study Design Timetable

Day:	1-21	22	23-27	28	29-30	31
Procedure	Dosed once daily; daily vaginal smear	Dosed; vaginal smear; ovariectomies	Dosed once daily	Dosed; 1 hr later, Estradiol implant	Dosed once daily	Dosed; collected postdose blood samples

2. **Animal assignment:** Rats were assigned to treatment groups as seen in Table 2 using a computerized block randomization procedure.

TABLE 2. Animal Assignment

Test Group	Dose to animal (mg/kg/day)	Number of females
Control	-----	40
Simazine		
Low (LDT)	2.5	20
Mid (MDT)	5	20
Mid-High (MHDT)	40	20
High (HDT)	200	20
DACT		
Low (LDT)	2.5	20
Mid (MDT)	5	20
Mid-High (MHDT)	40	20
High (HDT)	200	20
Atrazine		
Low (LDT)	2.5	20
Mid (MDT)	5	20
Mid-High (MHDT)	40	20
High (HDT)	200	20

3. **Dose selection rationale:** The dose levels were selected based on the results from a 28-day study (MRID 43934406) on the effects of atrazine on LH surge where diet administration of up to 200 mg/kg/day resulted in an attenuation of the LH surge and disruption of the estrous cycle.

4. **Dosage preparation and analysis:** Dosing formulations were prepared at least weekly by mixing appropriate amounts of test substance (simazine, DACT, or atrazine) with 0.5% carboxymethylcellulose. Two samples were taken from each dose preparation. One sample immediately was stored frozen at approximately -10 to -30° C; the other sample was stored at room temperature for 10 days, then stored frozen at approximately -10 to -30° C. Approximately 2.5 years after study initiation, these samples were analyzed for homogeneity (top, middle, and bottom) and weekly dose concentration analysis.

Results - Homogeneity Analysis (low and high dose formulations):

Simazine:	98.1% - 102% target
DACT:	100% - 105% target
Atrazine:	100% - 102% target

Concentration Analysis:

Simazine:	88.2% - 106% target
DACT:	79.8% - 110% target
Atrazine:	88.9% - 105% target

Dose analysis was performed on dose formulation samples that had been retained and frozen for approximately 2.5 years. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

5. **Ovariectomies and Estradiol Administration:** After 3 weeks of treatment, the rats were ovariectomized using aseptic surgical procedures. Six days after ovariectomies were performed, estradiol (4 mg/ml) was administered via a subcutaneously surgically implanted capsule, approximately 1 hour after the daily dose of test material or vehicle.

6. **Blood collection, sacrifice, and LH measurements:** Three days after the estradiol implant, and following dosing the usual daily dose, each rat was bled at six intervals (1300, 1600, 1800, 2000, 2200, 2400). Following the last bleed, all rats were sacrificed using O2/CO2 anesthesia followed by exsanguination. Blood samples were stored and later analyzed for lutenizing hormone (LH).

C. OBSERVATIONS

1. **Determination of cycling:** Daily vaginal smears (conducted in the morning) were taken beginning on Day 1 and continued for approximately 3 weeks (until the day of ovariectomies). Estrous cycle stages were not analyzed at the time of report finalization, and, therefore, are not included in this report.

2. **Clinical observations:** The rats were observed twice daily for evidence of mortality, morbidity, abnormal behavior and any other abnormal finding.

3. **Body Weights:** Individual body weights were recorded at randomization, once prior to treatment (Day 1), weekly thereafter, at the time of the estradiol implant, and on the day prior to sacrifice.

4. **Postmortem observations:** After the collection of the final blood sample, all surviving rats were sacrificed and a limited necrospy was performed. The necropsies were limited to examination of the rats for masses in the mammary region, uterus, vagina, and pituitary.

D. DATA ANALYSIS

1. Statistical analyses: In this study each test material was administered on a mg/kg/day basis. For purposes of comparison between compounds, the body weight change and LH values were adjusted on a molar equivalent basis relative to atrazine. Data were analyzed using Statistical Analysis System(SAS). Body weight and mean body weight change were analyzed using Analysis of Variance (ANOVA). The mean adjusted peak LH surge values were analyzed using repeated measures of analysis of variance. To address some of the limitations of the original analyses, statistical analyses of maximum increase in LH over baseline levels (LHMax), hour at which peak surge of LH occurred (TimeMax), and area-under-curve measure for the LH versus time profile (AUC) were performed using Dunnett's t test. Statistical evaluation of LHMax, TimeMax, and AUC allowed the inclusion of all animals and required no adjustments for the dose molarity of simazine and DACT (relative to atrazine).

2. Historical control data: Historical data were not provided.

II. RESULTS

1. Mortality: There were no compound-related deaths. Three rats in the control group died during the study, with two of those death in week 5. One rat in each simazine group died during week 5. Two rats in the DACT groups (5.0 and 200 mg/kg/day) died during week 5 and one during week 3 (40 mg/kg/day). In the atrazine group, one rat died during week 5 and one died during week 1. Some of the unscheduled deaths occurred during the blood sample collections at the end of the study and were found to not be treatment related. None of the unscheduled deaths occurred in the 4th week of the study during which surgical procedures were performed. The study report did not describe the cause of death.

2. Clinical signs: There were no clinical signs of toxicity that could be attributed to compound exposure.

3. Body weight and food consumption: Reported body weights (unadjusted) and body weight change (unadjusted and adjusted) are summarized in Tables 3 and 4. Mean body weight in the high dose group (200 mg/kg/day) for all three chemicals was significantly lower than control throughout the study, with the exception of DACT treated animals on day 15 (Table 3). Mean body weight in DACT high dose group was significantly lower than the mean body weight of simazine high dose group, and atrazine low dose group (2.5 mg/kg/day) had a significantly lower mean body weight than simazine low dose group.

During the first week of treatment there was a significant loss of mean unadjusted body weight at the 40 mg/kg/day and 200 mg/kg/day dose levels for all three chemicals, whereas the control rats exhibited a body weight gain (Table 4). When body weight was adjusted, with the exception of 40 mg/kg/day atrazine treated rats, the treatment related effects on mean adjusted body weight gain during the first week remained statistically significant. Statically significant treatment-related decreases in mean overall unadjusted and adjusted body weight gain (Days 1-29), relative to control, were noted for simazine and DACT treated rats at the 40 and 200 mg/kg/day dose levels, and for atrazine treated rats at the 200 mg/kg/day dose level.

Food consumption data were not included in this report.

TABLE 3. Mean (±SD) Body Weight[a]

Dose group	Day						
	1	8	15	22	28	29	31
Control							
0	247.8±17.5	249.9±19.8	257.0±20.1	267.6±20.3	284.2±21.4	284.1±20.0	275.1±19.5
Simazine							
2.5	248.2±20.2	253.4±21.1	264.1±22.6	271.8±22.9	291.8±22.6	291.2±23.4	280.9±24.6
5	248.6±22.5	251.3±19.6	260.9±22.6	269.5±21.0	286.2±23.2	285.7±22.9	275.4±22.1
40	249.8±20.2	247.1±19.6	256.4±22.3	260.5±22.0	279.5±23.6	279.4±22.8	270.9±23.8
200	244.9±20.2	237.5±17.2*	245.1±19.9*	249.4±16.7*	265.1±15.8*	265.5±15.5*	256.6±16.1*
DACT							
2.5	244.8±17.7	247.7±20.3	257.7±17.3	263.9±17.5	280.1±21.8	281.4±21.9	272.0±19.9
5	244.3±17.9	246.0±17.0	253.5±19.3	261.3±18.4	278.7±19.1	276.6±18.5	268.5±19.4
40	249.3±17.2	246.0±14.2	253.1±17.7	261.1±17.0	277.3±17.7	274.4±21.2	267.3±20.3
200	246.2±17.7	238.8±10.7*	249.6±17.0	247.8±18.4*	249.9±21.7*-	249.9±18.9*-	244.0±16.2*
Atrazine							
2.5	242.0±17.9	243.0±18.2	251.2±15.9+	258.0±17.9+	273.7±19.5+	274.1±19.4+	264.8±19.4+
5	246.8±16.5	249.0±16.9	260.7±18.1	268.6±19.5	284.7±16.6	284.7±17.5	272.6±15.9
40	244.3±17.3	243.2±21.2	252.1±20.9	258.9±21.4	274.6±22.7	276.4±23.7	266.2±22.2
200	247.1±18.2	236.1±18.1*	241.0±20.5*	247.7±20.4*	261.5±22.7*	261.4±23.0*	249.9±22.1*

[a] Data obtained from pages 52-53 in the study report.

* Statistically different from control, p<=0.05; −Statistically different from 200 mg/kg/day simazine (group 5) at p<=0.05; + Statistically different from 2.5 mg/kg/day simazine (group 2) at p<=0.05

TABLE 4. Mean (±SD) Unadjusted and Adjusted (in brackets) Body Weight Change [a]

Dose group	Day					
	1-7	8-14	15-21	22-28	22-29	1-29
Control						
0	2.1±11.6	7.1±6.9	9.2±7.7	16.6±8.2	16.1±8.0	36.0±12.2
Simazine						
2.5	5.3±7.8 [5.0]	10.7±6.1 [10.5]	7.7±5.7 [7.8]	20.0±7.2 [19.7]	19.4±8.1 [19.1]	43.0±13.1 [42.5]
5	2.8±9.1 [2.7]	9.6±5.5 [9.4]	8.7±4.1 [8.7]	16.7±5.9 [16.6]	16.2±5.6 [16.2]	37.2±10.2 [37.1]
40	-2.7±9.0* [-2.4]*	9.3±8.0 [9.3]	4.2±8.5* [4.5]*	19.0±7.1 [18.8]	18.9±7.7 [18.7]	29.6±12.1* [30.0]*
200	-7.4±9.7* [-6.8]*	7.6±8.5 [7.5]	4.3±7.1* [4.6]	15.8±7.5 [15.8]	16.1±7.8 [16.1]	20.6±10.1* [21.6]*
DACT						
2.5	2.9±8.1 [2.6]	10.1±8.3 [9.1]	6.2±5.8 [7.1]	16.3±8.0 [16.4]	17.6±8.1 [17.1]	36.6±9.1 [36.4]
5	1.7±5.4 [1.8]	7.5±7.6 [7.4]	7.8±6.4 [8.2]	17.4±4.2 [17.1]	16.2±5.5 [16.2]	32.4±9.8 [33.6]
40	-3.3±9.1* [-1.5]*	7.1±10.4 [7.1]	6.6±4.3 [7.4]	16.2±4.8 [16.3]	13.3±8.1' [14.2]'	25.5±16.5* [28.9]*
200	-7.4±14.8* [-4.3]*	10.8±12.7 [9.6]	-1.8±8.9*- [1.8]*	2.1±15.9*- [6.8]*-	2.5±8.0*- [6.9]*-	3.3±18.5* [13.9]*
Atrazine						
2.5	1.0±6.1 [1.0]+	8.3±4.2 [8.3]	6.8±5.2 [6.8]	15.7±4.5+ [15.7]	16.1±5.7 [16.1]	32.1±8.4+ [32.1]
5	2.2±12.3 [2.2]	11.7±6.9 [11.7]	7.9±8.3 [7.9]	16.2±7.8 [16.2]	16.2±8.2 [16.2]	38.0±11.2 [38.0]
40	-1.3±8.2* [-1.3]	8.8±6.0 [8.8]	6.9±6.0 [6.9]	15.7±5.8 [15.7]	17.4±6.2 [17.4]	31.8±12.3 [31.8]
200	-11.0±13.2* [-11.0]*	4.9±12.3 [4.9]	6.8±15.2' [6.8]	13.8±8.2' [13.8]'	13.7±8.7' [13.7]'	14.3±16.0* [14.3]*-

[a] Data obtained from pages 54-57

* Significantly different from control group at p <= 0.05; + Significantly different from group 2 at p <= 0.05; - Significantly different from group 5 at p <= 0.05; ' Significantly different from group 4 at p<= 0.05; ' Significantly different from group 9 at p <= 0.05

4. **Anatomical pathology**: There were no treatment-related findings.

5. **Plasma LH concentrations and LH surge:** Most animals exhibited relatively lower plasma LH concentrations at the baseline (1300 hour) blood collection relative to 1600, 1800, 2000, and 2400 hour blood collection intervals (Table 5). For most animals the peak LH concentration occurred at the 1800 hour interval. Plasma LH in all groups, including control, was higher at 2400 than plasma LH at 2200.

Results from initial pairwise comparison between groups showed that all three compounds had similar effects on diminishing the LH surge, as measured by adjusted peak LH (Table 6). All three compounds at the two highest doses, 40 and 200 mg/kg/day, significantly decreased LH surge (based on adjusted LH peak).

The evaluation of LHMax, TimeMax, and AUC indicated that simazine and DACT significantly decreased LHMax and AUC at 200 mg/kg/day; simazine also decreased LHMax and AUC at 40 mg/kg/day (Table 7). There were no significant differences between the atrazine-treated group and the control at any dose effect of treatment for LHMax or AUC. There was no effect of treatment with any of the three compounds on TimeMax. LHMax and AUC were positively correlated with each other whereas there was no association between LHMax and TimeMax. Results suggest that the higher doses were associated with lower values of LHMax and AUC; however there was no association between dose and TimeMax.

TABLE 5. Group Mean (± SD) Unadjusted and Adjusted (in brackets) Plasma LH (ng/ml) [a]

Dose group	Time					
	1300	1600	1800	2000	2200	2400
Control						
0	0.47±.60	2.13±1.95	3.38±2.70	2.12±1.64	0.42±.56	3.65±2.18
Simazine						
2.5	0.62±.70 [0.61±.66]	1.21±.82 [1.27±..76]	2.63±2.44 [2.68±2.29]	2.63±3.49 [2.60±3.26]	0.83±1.50 [0.80±1.41]	4.32±2.72 [4.28±2.55]
5	0.33±.38 [0.34±.35]	1.44±1.54 [1.49±.34]	1.99±1.87 [2.08±1.75]	1.57±1.74 [1.61±1.63]	0.42±.85 [0.42±.79]	3.15±1.36 [3.18±1.27]
40	0.70±.60 [0.68±.56]	1.17±1.06 [1.23±.99]	2.06±.94 [2.14±3.36]	0.94±1.13 [1.02±1.06]	0.51±.72 [0.50±.67]	3.22±1.75 [3.24±1.63]
200	0.71±.72 [0.69±.67]	0.74±.82 [0.83±.77]	1.27±1.57 [1.40±1.47]	0.82±1.05 [0.90±.98]	0.64±1.50 [0.62±1.40]	3.11±2.44 [3.15±2.28]
DACT						
2.5	0.29±.39 [0.35±.26]	1.60±1.48 [1.77±1.00]	2.62±1.89 [2.87±1.28]	1.53±1.26 [1.72±.85]	0.26±.35 [0.31±.23]	3.83±2.74 [3.77±1.85]
5	0.47±.63 [0..47±.42]	2.04±2.71 [2.07±1.83]	2.79±3.62 [3.06±2.41]	1.73±1.64 [1.86±1.11]	0.32±.40 [0.38±.28]	2.85±1.71 [3.11±1.16]
40	0.34±.41 [0.38±.28]	1.14±.88 [1.46±.59]	2.13±1.93 [2.54±1.30]	1.86±1.77 [1.95±1.20]	0.40±.53 [0.41±.36]	3.30±1.91 [3.41±1.29]
200	0.17±.37 [0.27±.25]	0.08±.03 [0.75±.02]	0.27±.29 [1.28±.20]	0.98±1.34 [1.35±.91]	0.69±1.48 [0.60±1.13]	3.38±1.67 [3.47±1.12]
Atrazine						
2.5	0.35±.42	2.04±1.49	2.95±2.42	1.51±1.80	0.16±.24	2.97±2.70
5	0.47±.49	1.71±2.38	2.73±2.53	2.02±1.94	0.38±.50	2.46±1.55
40	0.62±.72	1.56±1.67	1.76±1.60	1.29±1.28	0.57±1.12	2.74±1.62
200	0.30±.40	0.70±1.05	1.84±2.26	1.76±2.25	0.63±.80	2.82±1.56

[a] Data obtained from pages 423-449

Table 6. Mean (± SD) Peak LH Range and Adjusted (in brackets) Peak LH Range[ab]

Dose group	Peak and Adjusted Peak LH Range		
	Pre-Peak	Peak	Post-Peak
Control			
0	1.75±1.59	4.10±2.52	2.19±1.81
Simazine			
2.5	1.55±2.11 [1.64]	3.75±3.44 [3.70]	1.91±1.93 [1.87]
5	1.10±1.34 [1.16]	2.43±2.09 [2.46]**	1.32±1.33 [1.37]
40	1.03±.91 [1.10]	2.07±1.20 [2.12]**	0.77±.68 [0.84]
200	0.65±.79 [0.79]	1.78±1.64 [1.94]**	0.79±1.01 [0.92]
DACT			
2.5	1.54±1.34 [1.68]	3.00±1.73 [2.97]*	1.23±1.19 [1.50]
5	1.63±2.49 [2.02]	3.30±3.41 [3.14]	1.45±2.05 [1.54]
40	1.28±1.39 [1.48]	2.77±1.99 [2.78]*	0.89±.77 [1.39]
200	0.28±.29 [0.98]	1.00±1.04 [1.45]**	0.47±.71 [0.80]
Atrazine			
2.5	1.68±1.49	3.51±2.41	1.52±1.43
5	2.05±2.56	3.53±2.57	1.34±1.30
40	1.08±.91	2.66±1.71*	1.61±1.71
200	1.41±2.06	2.75±2.43*	1.25±1.15

[a] Data obtained from pages 451-477

[b] Included only surviving animals with sufficient plasma estradiol concentration

* Statistically different from control, $p < 0.05$

** Statistically different from control, $p < 0.01$

Table 7. LH Surge Effects Measures (Mean ± SE)[ab]

Dose group	LH Surge Effects Measures			
	N	LHMax (ng/ml)	AUC (hrs-ng/ml)	TimeMax (hrs)
Control				
0	37	3.49±0.43	13.46±1.98	18.16±0.27
Simazine				
2.5	20	2.94±0.78	9.7±3.28	17.90±.51
5	18	2.1±.49	8.71±2.53	17.78±.32
40	18	1.37±.27*	2.77±1.52*	17.89±.57
200	20	1.33±.44*	1.33±1.67*	17.25±.64
DACT				
2.5	20	2.72±.39	10.41±1.81	18.30±.30
5	19	2.84±.70	10.94±3.22	18.21±.34
40	18	2.43±.47	8.70±1.85	18.56±.32
200	18	0.91±.29*	2.11±.71*	18.72±.71
Atrazine				
2.5	20	2.99±.55	11.54±2.25	17.45±.38
5	20	2.84±.53	10.63±2.81	18.25±.42
40	17	2.01±.40	6.08±2.01	17.94±.55
200	19	2.11±.54	7.29±2.31	18.47±.51

[a] Data obtained from pages 58; 525.

[b]Analysis excluded animals that died or had low estradiol levels; the analysis were based on unadjusted plasma LH measurements.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author found that over the duration of the study significant decreases in body weight gains were noted at the 200 mg/kg/day dose level for simazine, DACT, and atrazine, with significant weight losses during the 1st week of the study. Initial analyses of the effects of the three compounds on LH surge found that all three compounds had similar effects on diminishing the LH surge at the higher doses. As shown in Table 6, Simazine, DACT, and atrazine at 40 and 200 mg/kg/day all significantly decreased the LH surge (based on adjusted LH peak). LH surge was

also significantly decreased at the simazine 5 mg/kg/day and DACT 2.5 mg/kg/day dose levels.

Additional analyses that required no adjustment of molarity showed that simazine and DACT significantly decreased LHMax and AUC at 200 mg/kg/day and that simazine also decreased LHMax and AUC at 40 mg/kg/day (Table 7). There were no significant differences between the atrazine-treated group and the controls at any dose effect of treatment for LHMax or AUC. The data do suggest that there is a dose-response relationship for atrazine, simazine, and DACT for both LHMax and AUC. There was no effect of treatment with any of the three compounds on TimeMax. LHMax and AUC were positively correlated with each other whereas there was no association between LHMax and TimeMax.

The author of this study concluded that the LOAEL for systemic toxicity is 40 mg/kg/day for simazine, DACT, and atrazine, based on body weight effects. The NOAEL for all three compound is 5 mg/kg/day. The LOAEL for endocrine effects of simazine is 40 mg/kg/day, based on decreased LHMax and AUC. The NOAEL for endocrine effects of simazine is 5 mg/kg/day. The LOAEL for endocrine effects of DACT is 200 mg/kg/day, based on decreased LHMax and AUC. The NOAEL for endocrine effects of DACT is 40 mg/kg/day. The LOAEL for endocrine effects of atrazine is greater than 200 mg/kg/day. The NOAEL for endocrine effects of atrazine is not demonstrated.

B. REVIEWER COMMENTS: This study evaluated the effects of simazine, DACT, and atrazine on LH surge in female Sprague-Dawley Crl:CD BR rats. There were no treatment-related deaths, although a number of deaths, including in the control group, occurred during week 5 of the study. These deaths were likely a result of technique errors rather than treatment-related. Body weight and body weight gain were adversely affected in all three treatment groups. Mean body weight in the high dose group (200 mg/kg/day) for all three chemicals was significantly lower than control throughout the study, with the exception of DACT treated animals on Day 15. Mean overall body weight gain (unadjusted and adjusted) was significantly lower throughout the study for simazine and DACT treated rats at the 40 and 200 mg/kg/day dose levels and for atrazine treated rats at the 200 mg/kg/day dose level. With the exception of adjusted body weight for 40 mg/kg/day atrazine treated rats, a significant loss in mean body weight (unadjusted and adjusted) occurred during the first week of the study in all treatment groups at 40 mg/kg/day and 200 mg/kg/day. The effect of food consumption on body weight and body weight gain can not be determined because the data were not provided. However, in a previous study (MRID 43934406) on the effects of atrazine in the diet on LH surge, food consumption was significantly decreased in the 40 and 200 mg/kg/day dose groups.

Results of this study also showed that for most animals the peak LH concentration occurred at the 1800 hour interval and that plasma LH in all groups, including control, was higher at 2400 than plasma LH at 2200. Higher plasma LH at the terminal blood collection may have been due to the procedures used to collect the blood. The first five blood samples were taken from the jugular vein of unanesthetized rat, while the last blood sample was taken from the vena cava of anesthetized rats. Thus, the higher plasma LH at last blood collection was not found to be treatment-related.

The effects of simazine, DACT, and atrazine on LH surge were analyzed by two different methods. The investigators of this study initially conducted analyses on LH surge using the adjusted peak LH concentrations of those animals that followed the appropriated peak LH surge patterns (i.e. those animals

with estradiol levels high enough to produce an appropriate LH surge). These results showed that simazine, DACT, and atrazine had similar effects on diminishing the LH surge at the higher doses. Simazine, DACT, and atrazine at 40 and 200 mg/kg/day all significantly decreased the LH surge. Analyses of pre-peak, peak, and post-peak LH concentrations, and comparison of responses between compounds (at the same dose levels) supported these results.

The investigators also presented three additional measures that included data from all animals and required no adjustments for the dose molarity of simazine and DACT (relative to atrazine): maximum increase in LH over baseline levels (LHMax), hour at which peak surge LH occurred (TimeMax), and area-under-curve measure for the LH vs. time profile (AUC). The evaluation of LHMax, TimeMax, and AUC indicated that simazine and DACT significantly decreased LHMax and AUC at 200 mg/kg/day; simazine also decreased LHMax and AUC at 40 mg/kg/day. There were no significant differences between the atrazine-treated group and the control at any dose effect of treatment for LHMax or AUC. There was no effect of treatment with any of the three compounds on TimeMax. LHMax and AUC were positively correlated with each other whereas there was no association between LHMax and TimeMax. Results suggested that the higher doses were associated with lower values of LHMax and AUC; however there was no association between dose and TimeMax. The correlation between attenuation of the LH surge and disruption of estrous cycle in this study can not be determined because estrous cycle data were not reported.

In contrast to the author's conclusions, the reviewer based the NOAEL and LOAEL on the effects of simazine, DACT, and atrazine on peak plasma LH concentrations. This measure was selected because peak plasma LH is the standard for measuring LH surge. Using LHMax, AUC, and TimeMax subject the data to manipulation and does not allow the comparison of the current results to previously submitted studies on atrazine's effects on the LH surge. In addition, and more importantly, regardless of which measures are utilized the results show that simazine and DACT suppress LH surge just as atrazine has been found to do in previous reports. In fact, the results from the effects of atrazine on peak LH values are consistent with the previous studies on the effects of atrazine on LH surge, while the compound's effects on LHMax and AUC are not. **Therefore, the reviewer concludes that the The LOAEL for endocrine effects of atrazine, simazine, and DACT is 40 mg/kg/day, based on decreased adjusted peak LH concentrations. The NOAEL for endocrine effects is 5 mg/kg/day. The LOAEL for systemic toxicity is 40 mg/kg/day for simazine, DACT, and atrazine, based on body weight effects, and the NOAEL for all three compounds is 5 mg/kg/day.**

C. <u>STUDY DEFICIENCIES</u>: Although estrous cycle data were collected, the effects of simazine, DACT, and atrazine on estrous cycle in this study could not be determined because the data were not submitted with this report. This lack of information, however, does not affect the interpretation of the results.

EPA Reviewer: Artensie Flowers, PhD, MPH
SIMB, Health Effects Division (7509C)
EPA Secondary Reviewer: _____
[Insert Branch], Health Effects Division (7509C)

Signature: _____
Date _____
Signature: _____
Date _____

Template version 11/01

TXR#: 0050653

DATA EVALUATION RECORD - SUPPLEMENT
See TXR#007240 for original review

STUDY TYPE: Combined chronic toxicity/carcinogenicity diet - Mice; OPPTS 870.4300 [§83-5]; OECD 453.

PC CODE: 080807

DP BARCODE: D274214
SUBMISSION NO.: S562293

TEST MATERIAL (PURITY): Simazine Technical (purity not reported)

SYNONYMS: 2 Chloro-4, 6 bis (ethylamino)-s-triazine

CITATION: Hazelette, J.R., Green, J.D.(1988). Simazine Technical: 95-week oral toxicity/oncogenicity study in mice. Pharmaceutical Div., Ciba-Geigy. Laboratory report number: 842121. April 4, 1988. MRID: 40614404. Unpublished.

SPONSOR: Ciba-Geigy, Greensboro, NC

EXECUTIVE SUMMARY:

In a carcinogenicity toxicity study (MRID 40614404), simazine (purity not reported, batch no. FL 840988) was administered to CD1 mice, 60/sex/dose, in the diet at dose levels of 0, 40, 1000, 4000 ppm (0, 0.5.3/6.2, 131.5/160, 542/652.1 mg/kg/day, males/females) for 95 weeks. For interim data, additional animals/dose were administered test material in the diet and sacrificed (10 animals per sacrifice) at week 26 or 52. In addition, ten animals in the control and 4000 ppm groups were sacrificed at week 56 after a 4-week recovery period. Sixty animals were also sacrificed at the start of the study to establish baseline parameters and 20 sentinel animals were sacrificed mid-way (52 weeks) into the study to check for viruses.

While there were many clinical observations reported during the study, no observation was determined to be an effect of dosing. Mortality was not significantly increased in treated groups vs controls.

Mean body weight and percent body weight gain were significantly reduced at various time points in both sexes of the 1000 ppm (MDT) and 4000 ppm (HDT) groups. The reductions at the highest dose were significant throughout most of the study and at the mid-dose the reductions

were significant in males beginning at week 24 and in females beginning at week 16. After 92 weeks of the study, HDT males and females body weights were significantly reduced by 12% and 20%, respectively, while MDT females body weights were significantly reduced by 8%. Mean food consumption was consistently decreased ($p< 0.05$, 7-16% decrease, depending on time point) in the HDT females from week 2 until the end of the study. Likewise, water consumption was deceased ($p<0.05$) in HDT females from week 2 onward.

Hematology and clinical chemistry parameters did not appear to be affected by exposure to simazine. Organ weights did not appear to be altered either. There were no increased incidences of neoplastic lesions in any dose group compared to controls.

The LOEL is 1000 ppm (131.5/160 mg/kg/day, males/females), based on decreases in body weight and percent body weight gain in both sexes, and decreases in food consumption in males. The NOEL is 40 ppm (5.3/6.2 mg/kg/day, males/females).

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls.

This combined chronic/oncogenicity study in mice is **Acceptable-Guideline** and satisfies the guideline requirement for a combined chronic/oncogenicity study [(OPPTS 870.4300); OECD 453] in mice.

COMMENTS: This is a revised Executive Summary with minor modifications. The LOEL was corrected to decreased food consumption based on **males** instead of females.

DER #2

Chemical Name: Simazine
18-Month Carcinogenicity Study in Mice
Sponsor Name: Ciba-Geigy
Year of Study: ~~1989~~ 1985
MRID No: 40614404
HED Doc. No. TXR 007240

CITATION: Hazelette, J.R., Green, J.D.(1988) Simazine technical: 95-week oral toxicity/oncogenicty study in mice. Pharmaceutical Div., Cibe-Geigy. Laboratory report number:842121. April 4, 1988. MRID:40614404. Unpublished.

EXECUTIVE SUMMARY:

In a carcinogenicity toxicity study (MRID 40614404), simazine, purity not reported, was administered to 480 CD1 mice, 60/sex/dose in the diet at dose levels of 40, 1000, 4000 ppm (0,5.3/6.2, 131.5/160, 542/652.1 mg/kg/day, males/females) for 95 weeks. Additionally, 10 animals at each dose were dosed and sacrificed at 26 and 52 weeks to obtain interim data. Ten animals at the control and 4000 ppm groups were dosed and sacrificed for 52 weeks then allowed 4 weeks recovery and sacrificed as a recovery group. These groups were referred to as satellite groups. Sixty animals were also sacrificed at the start of the study to establish baseline parameters and 20 sentinel animals were sacrificed mid-way (52 weeks) into the study to check for viruses.

While there were many clinical observations reported during the study, no observation was determined to be an effect of dosing. Mortality was not significantly increased in treated groups *vs* controls.

Mean body weight and percent body weight gain were significantly reduced at various time points in both sexes of the 1000 ppm(MDT)and 4000 ppm(HDT)groups. By 92 weeks into the study MDT male and female body weights were reduced 10 and 9% respectively while HDT male and female weights were reduced 12 and 20% respectively. Mean food consumption was consistently decreased ($p<0.05$, 7-16% decrease depending on time point)in the HDT females from week two until the studies end. Likewise, water consumption was deceased($p<0.05$)in HDT females from week 2 onward.

Hematology and clinical chemistry parameters did not appear to be affected by exposure to simazine. Organ weights did not appear to be altered either.

There were no increased incidences of neoplastic lesions in any dose group compared to controls.

The LOEL is 1000 ppm(131.5/160 mg/kg/day, males/females), based on decreases in body weight and percent body weight gain in both sexes, and decreases in food consumption in the females. The NOEL is 40 ppm(5.3/6.2 mg/kg/day,males/females).

At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls.

Author: Robert Hazelette 6/12/98

83-

EPA: 68D80056
DYNAMAC No. 1-10A
February 3, 1989

DATA EVALUATION RECORD

SIMAZINE

Chronic Toxicity/Oncogenicity Feeding Study in Mice

APPROVED BY:

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Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 2-3-89

EPA: 68D80056
DYNAMAC No. 1-10A
February 3, 1989

DATA EVALUATION RECORD

SIMAZINE

Chronic Toxicity/Oncogenicity Feeding Study in Mice

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DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity/Oncogenicity Feeding Study in Mice.

ACCESSION/MRID NUMBER: 406144-04.

TEST MATERIAL: Simazine technical.

SYNONYM(S): 2 Chloro-4,6 bis(ethylamino)-s-triazine.

STUDY NUMBER(S): Laboratory Study No. 842121.

SPONSOR: Agricultural Division, Ciba-Geigy Corp., Greensboro, NC.

TESTING FACILITY: Pharmaceuticals Division, Ciba-Geigy Corp., Summit, NJ.

TITLE OF REPORT: Simazine technical; 95-week oral toxicity/oncogenicity study in mice.

AUTHOR(S): Hazelette, JR and JD Green.

REPORT ISSUED: April 4, 1988.

CONCLUSIONS:

Simazine was not oncogenic in CD-1 mice when fed in the diet at concentrations of 40, 1000, or 4000 ppm for 95 weeks. There was a decrease in mean body weight in both males and females in the mid-and high-dose groups, and a decrease in food consumption in mid- and high-dose males and in mid-dose females. There were decreases in erythroid parameters which may have been related to weight loss. Other hematologic parameters were not affected. Clinical chemistry values and urinary parameters were normal in dosed groups. Organ-to-body weight ratios were increased in high-dose females for several organs; however, there were no histologic correlates and the changes were accompanied by decreased terminal body weights. There were no nonneoplastic changes related to dosing. The incidence of amyloidosis was high in all groups. The LOEL based on decreased weight gain was 1000 ppm and the NOEL 40 ppm.

Classification: Core guideline.

A. MATERIALS:

1. Test Compound: Simazine technical; description: white powder; batch No.: FL 840988; purity: not reported.

2. Test Animals: species: mice; strain: Crl:CD1(ICR)BR; age: approximately 5 weeks at initiation; weight: males--19.1 to 32.1 g; females--14.4 to 26.3 g; source: Charles River Breeding Laboratories, Kingston, NY.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated to laboratory conditions for 14 days and were assigned randomly by sex to the following test groups after passing a physical examination:

Test group	Dose in diet (ppm)	Main study (95 weeks)	Satellite groups (Pre)	(26 weeks)	(52 weeks)	(56 weeks)[c]
		Males/Females				
1 Control	0	60	-	10	10	10
2 Low (LDT)	40	60	-	10	10	-
3 Mid (MDT)	1000	60	-	10	10	-
4 High (HDT)	4000	60	-	10	10	10
5 Baseline[a]	0	-	60	-	-	-
6 Sentinel[b]	0	-	-	-	20	-

[a]Used for baseline laboratory values; 30/sex at -1 and at 2 weeks.
[b]Used for viral screen.
[c]Recovery group; received undosed diets from week 52 to 56.

Mice were housed individually in a temperature and humidity controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: Dietary mixtures of test substance at concentrations of 0, 40, 1,000, and 4,000 ppm were prepared and used within 21 days. Stability of test compound stored for 40 days at room temperature in closed amber glass containers was determined. Test Compound in the diets was analyzed at 4 week intervals for 1 year and at approximately 8-week intervals thereafter. Homogeneity was determined at weeks 1, 58 (high-dose), and 68.

   Results: The diets were homogeneous; the standard deviations as percent ranged from 0.2 to 2.3 percent for samples at 3 levels. Test material was stable in diets; 95 and 99% was recovered after 40 days storage at room temperature, at dietary levels of 40 and 4,000 ppm, respectively. All diets were within 8 percent of target. Table 1 presents representative analytical data.

3. Food and Water Consumption: Animals received food (Purina Rodent Chow No. 5002) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data:

   Body weights, food consumption, clinical pathology, and organ weights were analyzed by Bartlett's test for equality of variances. If variances were homogeneous, Dunnett's test was used to compare control versus each dose group. Rank transformations or nonparametric tests were used when variances were not homogeneous. Survival data were analyzed using Kaplan-Meir estimates. The generalized Wilcoxon test for equality and the Mantel-Cox log-rank test

were used for group comparisons. Pathology data were analyzed separately by sex using the Fisher exact test. In addition, tumor incidence was analyzed by time-adjusted analysis based on the Peto method.

TABLE 1. Analysis of Simazine in Test Diets at Representative Intervals

		Target Concentration (ppm)		
		40	1,000	4,000
Week				
1	Concentration (ppm)	37.5	999.0	3719
	Percentage of target	94	100	93
24	Concentration (ppm)	38.7	964.0	3970
	Percentage of target	97	96	99
52	Concentration (ppm)	40.3	1022	3952
	Percent of target	101	102	99
92	Concentration (ppm)	38.8	1030	4145
	Percentage of target	97	103	104

5. Quality Assurance: A quality assurance statement was signed and dated April 4, 1988.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for mortality and moribundity (once daily on weekends). Animals received detailed physical examinations, including palpations at initiation and at 2-week intervals during the study.

Results: There were no effects of dosing on the incidence of clinical signs. The most frequent observations were corneal opacity, cachexia, polyuria (males) and fur staining. Summary incidence data (observation in any animal in a group at any study interval) and group incidence for each type of observation at weekly intervals were presented. Examination of the latter tabulation (CBI Report, Table 8.4) indicated that all observations were incidental. There was a fairly high incidence of corneal opacity in control and high-dose animals at various intervals of the study. This may have been caused by periorbital bleeding for clinical pathology but this could not be verified in the absence of individual findings.

The initial viral screen on the sentinel animals indicated the presence of antibodies to MMV (mouse minute virus) but none of the other viruses tested positive. Since MMV was found in controls as well as dosed groups and since it did not adversely affect survival, it is not considered a serious consequence in the health of the mice.

There was no significant effect of dosing on survival. Table 2 presents data on mortality and survival.

TABLE 2. Cumulative Mortality and Percent Survival in Mice Fed Simazine for 95 Weeks

Dietary level (ppm)	No. of animals initial	No. of animals termination	No. of mortalities and (percent survival) at week 52	78	96
		MALES			
0	90	19	3(96)[a]	34(46)[b]	44(30)[b]
40	80	15	1(98)	32(50)	47(24)
1000	80	13	1(98)	35(43)	48(21)
4000	90	15	2(97)	28(54)	48(25)
		FEMALES			
0	90	20	3(96)	17(72)	35(43)
40	80	26	4(94)	21(65)	34(43)
1000	80	35	4(94)	14(76)	24(60)
4000	90	25	5(93)	17(72)	36(42)

[a]Percent survival was based on 80, 71, 70, and 80 males and 80, 70, 70, and 80 females at 0, 40, 1000, and 4000 ppm; 9 to 10 animals/group were sacrificed at 26 weeks.

[b]Percent survival based on 63, 62, 61, and 61 males and 61, 60, 60 and 61 females at 0, 40, 1000, and 4000 ppm; 9 to 10 animals were sacrificed at week 52 in all groups and at week 56, 8 control and 9 high-dose males and 10 control and 9 high-dose females in the recovery segment were sacrificed. These values differ slightly from Table 8.1 of the report which based survival on the total number of animals minus the animals scheduled for interim sacrifice.

2. Body Weight: Mice were weighed weekly from 1 week prior to initiation to week 13 and monthly from week 16 to study termination.

Results: Table 3 presents representative data on mean body weights in males and females. There was a significant reduction of mean body weights and percent weight gain in males and females receiving 1000 ppm and 4000 ppm. The reductions at the highest dose were significant throughout most of the study and at the mid dose the reductions were significant in males beginning at week 24 and in females beginning at week 16. The mean body weights of males receiving 40 ppm were slightly but significantly ($p < 0.05$) decreased at 4 study intervals (44, 56, 60, and 64 weeks). These were not considered of toxicological significance by

the study authors because they were isolated occurrences. At the termination of the 4-week recovery period, the mean body weight in the group of males that had previously received 4000 ppm simazine (42.7 g) did not differ significantly from controls (39.8 g) but in recovery females the mean body weights still remained depressed in the group previously received 4000 ppm simazine (27.8 g compared to 38.6 g).

TABLE 3. Representative Results of Mean Body Weights of Mice Fed Simazine Technical For 95 Weeks

Dose group (ppm)	Mean body weights (g ± S.E.) at day					
	0	7	140	392	504	644
			MALES			
0	23.9 ± 0.18	26.7 ± 0.20	38.6 ± 0.39	42.5 ± 0.61	42.6 ± 0.95	41.0 ± 1.65
40	24.2 ± 0.22	27.2 ± 0.23	38.7 ± 0.47	40.5 ± 0.54*	40.8 ± 0.64	39.3 ± 0.66
1000	23.9 ± 0.22	26.6 ± 0.24	36.9 ± 0.43**	39.3 ± 0.57**	38.2 ± 0.74**	38.8 ± 1.33
4000	24.2 ± 0.19	25.9 ± 0.21*	34.8 ± 0.30**	36.8 ± 0.40**	36.8 ± 0.45**	36.0 ± 0.99*
			FEMALES			
0	20.0 ± 0.17	21.9 ± 0.16	32.5 ± 0.40	36.6 ± 0.63	37.4 ± 0.71	37.5 ± 0.88
40	20.3 ± 0.20	21.8 ± 0.18	32.4 ± 0.39	36.6 ± 0.64	36.9 ± 0.65	37.1 ± 0.98
1000	20.2 ± 0.18	21.5 ± 0.17	30.2 ± 0.27**	33.7 ± 0.42**	34.2 ± 0.50**	34.4 ± 0.60**
4000	20.5 ± 0.17	20.8 ± 0.16**	27.9 ± 0.22**	29.2 ± 0.32**	30.4 ± 0.41**	30.0 ± 0.52**

*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

3. Food Consumption and Compound Intake: Consumption was determined and mean daily diet consumption was calculated at the same intervals as the weighings. Compound intake was calculated. Water consumption was measured for 5 days at weeks 1, 2, 52, 53, 92, and 93.

Results: Mean food consumption was decreased compared to controls in males and females receiving 4000 ppm and in males receiving 1000 ppm. The decreases were significant at most intervals to 84 weeks. Table 4 summarizes representative data. In the recovery groups, the food consumption also correlated with body weight gains; it was decreased compared to controls in the females but not males that had previously received 4000 ppm simazine. Mean compound intake for the entire study was 5.3, 131.5, and 542 mg/kg/day for males receiving 40, 1000, and 4000 ppm, respectively; for females at those doses intake was 6.2, 160.0 and 652.1 mg/kg/day, respectively.

TABLE 4. Representative Food Consumption for Mice Fed Simazine Technical For 95 Weeks

Dose group (ppm)	Mean food consumption (gm/week ± S.E.) at day 7	14	84	196	364	644
			MALES			
0	48.1 ± 0.76	44.8 ± 0.47	43.0 ± 0.82	34.0 ± 0.43	28.9 ± 0.37	33.3 ± 1.0
40	48.9 ± 0.86	48.0 ± 0.56*	43.3 ± 0.82	32.8 ± 0.44	28.2 ± 0.45	32.4 ± 1.0
1000	48.5 ± 0.75	39.7 ± 0.63**	39.4 ± 0.62**	31.7 ± 0.47**	27.6 ± 0.36*	32.4 ± 1.4
4000	47.4 ± 0.84	38.7 ± 0.51**	41.5 ± 0.87	30.2 ± 0.35**	27.4 ± 0.34**	31.2 ± 0.9
			FEMALES			
0	45.2 ± 0.99	43.07 ± 0.52	47.7 ± 0.87	33.9 ± 0.70	29.9 ± 0.59	32.6 ± 0.98
40	46.5 ± 0.80	44.9 ± 0.70	46.9 ± 0.90	34.1 ± 0.77	28.7 ± 0.51	32.2 ± 0.62
1000	47.4 ± 0.97	43.8 ± 0.79	44.3 ± 0.86*	32.2 ± 0.78	28.1 ± 0.61	32.4 ± 0.79
4000	44.1 ± 0.68	36.1 ± 0.38**	44.5 ± 0.76*	30.9 ± 0.75*	27.9 ± 0.60*	29.6 ± 0.92*

*Significantly different from control values at p <0.05.
**Significantly different from control values at p <0.01.

Water consumption tended to be decreased in mid- and high-dose males and females (Table 5).

TABLE 5. Representative Water Consumption for Mice Fed Simazine Technical for 95 Weeks

Dose group (ppm)	Mean water consumption (gm/week ± S.E.) at week 1	2	52	92
		MALES		
0	40.1 ± 2.3	45.2 ± 2.1	33.6 ± 2.2	43.3 ± 4.3
40	41.6 ± 2.4	48.1 ± 2.5	35.1 ± 3.6	34.2 ± 5.0
1000	35.2 ± 2.6	38.1 ± 3.0	29.9 ± 3.5	34.4 ± 3.3
4000	32.3 ± 1.8	35.1 ± 2.6*	28.9 ± 2.4	33.4 ± 3.2
		FEMALES		
0	35.4 ± 2.1	36.3 ± 1.8	38.5 ± 7.2	43.3 ± 6.4
40	34.9 ± 2.2	35.5 ± 2.1	31.0 ± 3.2	33.7 ± 3.1
1000	30.5 ± 1.3	31.7 ± 1.7	30.1 ± 3.9	30.4 ± 2.8
4000	28.5 ± 1.5*	23.4 ± 1.1**	21.7 ± 2.0**	26.9 ± 3.7*

*Significantly different from control values at p <0.05.
**Significantly different from control values at p <0.01.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on all animals prior to initiation and all survivors at week 52 and prior to termination (week 96). Examination was also performed on mice in the recovery groups prior to sacrifice (week 56) and on 3 to 6 males/group and 5 to 9 females/group at week 78.

Results: There were no abnormalities at the predose examination. There were no apparent increases in the incidence of findings in dosed groups when compared to controls. Table 6 summarizes findings at weeks 52 and 95.

TABLE 6. Representative Ophthalmologic Findings in Mice Fed Simazine Technical for 95 Weeks

Finding	Dose group (ppm) 0	40	1000	4000
	Week 52			
Corneal opacity				
Males	20/77[a]	10/67	13/67	18/75
Females	7/76	4/66	10/66	7/54
	Week 95			
Corneal opacity				
Males	5/19	8/15	1/13	3/15
Females	2/27	1/26	3/36	1/29
Cataract				
Males	6/19	10/15	2/13	10/15
Females	18/27	10/26	17/36	17/29

[a]The numerator is the number of animals with the finding and the denominator the number examined.

5. Hematology and Clinical Chemistry: Blood was collected from the periorbital sinus prior to study initiation and at 6 and 12 months for hematology and clinical analysis from 10 animals/sex/group and prior to termination on all survivors. An additional group of 60 mice/sex were sacrificed during week -1 and 2 to obtain baseline clinical laboratory values. The CHECKED (X) parameters were examined:

a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation:thromboplastin time (PT)-(baseline only)

Blood smears were prepared for all animals that were sacrificed moribund for differential white cell counts and microscopic evaluation of red cell morphology.

Results: Table 7 summarizes selected data on hematology. Erythrocyte counts (RBC) tended to be decreased in the high-dose groups at all intervals. The decreases were slight and values were not consistently significant at all intervals. Hematocrit (HCT) and hemoglobin (HGB) values tended to be decreased at the high dose but the values were only significant for HCT for high-dose males at 184 day and for HGB in high-dose females at 365 days. There were no clear cut dose-related trends and the changes in erythroid indices (MCV, MCHC) did not correlate with changes in RBC, HCT and HGB. Slight alterations in other hematologic parameters were not considered of any biologic importance. No Heinz bodies were found. Data on blood smears for animals sacrificed moribund were not useful because of frequent technical problems and poor smears. Only a few slides could be evaluated. Baseline data were not reported.

*Recommended by Subdivision F (October 1982) Guidelines.

TABLE 7. Selected Hematology Parameters (Mean ± S.E.) in Male Rats Fed Simazine Technical for 95 Weeks

Parameter/Interval	Dietary level (ppm) 0	40	1000	4000
		MALES		
RBC (10^6/mm^3)				
184 days	8.98 ± 0.20	8.11 ± 0.28	7.57 ± 0.28**	8.11 ± 0.27*
365 days	8.10 ± 0.23	7.68 ± 0.54	7.89 ± 0.22	7.81 ± 0.18
667 days	6.63 ± 0.21	6.95 ± 0.20	6.64 ± 0.18	5.96 ± 0.15*
HGB (g/dL)				
184 days	15.50 ± 0.37	15.30 ± 0.24	14.68 ± 0.22	14.55 ± 0.30
365 days	15.16 ± 0.30	14.18 ± 0.85	14.39 ± 0.34	14.62 ± 0.25
667 days	12.94 ± 0.47	13.73 ± 0.34	13.46 ± 0.36	12.23 ± 0.30
HCT (%)				
184 days	48.60 ± 1.08	47.00 ± 0.98	45.70 ± 0.83	44.30 ± 0.82*
365 days	45.67 ± 0.78	43.11 ± 2.16	43.22 ± 0.91	43.50 ± 0.91
667 days	39.74 ± 1.45	41.69 ± 1.04	41.15 ± 1.32	37.13 ± 0.82
		FEMALES		
RBC (10^6/mm^3)				
184 days	8.04 ± 0.33	8.64 ± 0.18	8.30 ± 0.23	7.76 ± 0.32
365 days	8.86 ± 0.46	8.45 ± 0.29	7.82 ± 0.21	7.77 ± 0.24*
667 days	6.46 ± 0.27	7.14 ± 0.16	5.89 ± 0.17	5.83 ± 0.17
HGB (g/dL)				
184 days	15.84 ± 0.28	15.41 ± 0.17	15.57 ± 0.24	15.24 ± 0.27
365 days	16.98 ± 1.52	15.43 ± 0.29	14.38 ± 0.29	14.15 ± 0.31*
667 days	13.42 ± 0.50	14.26 ± 0.20	12.43 ± 0.35	12.43 ± 0.25
HCT (%)				
184 days	47.70 ± 0.72	47.80 ± 0.47	47.00 ± 0.58	46.00 ± 0.54
365 days	50.22 ± 3.70	45.50 ± 0.85	42.60 ±0.79	41.80 ± 0.76
667 days	41.50 ± 1.51	43.50 ± 0.62	38.24 ± 1.10	37.92 ± 0.65

*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
	Magnesium*		Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Cholesterol*
X	Sodium*	X	Globulins
		X	Glucose*
	Enzymes	X	Total bilirubin*
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase	X	Total protein*
X	Creatinine phosphokinase*		Triglycerides
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)*		
X	Serum aspartate aminotransferase (SGOT)*		
X	Gamma glutamyltransferase (GGT)		
	Urea		

Results: There were no compound-related changes in any serum chemistry parameter. A few values that were significantly different from controls were sporadic, not consistent between intervals of analysis or dose-related, and were marginally changed and within the range of the concurrent controls. These changes included an increase in albumin and chloride in mid- and high-dose females at day 184 and a decrease in LDH in mid-dose females at day 365.

6. Urinalysis: Urine was collected from 10 animals/sex/group at 27, 53, and 96 weeks and from control and high-dose animals in the recovery groups at the beginning of week 57.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
	Sediment (microscopic)*		Nitrate
	Protein*	X	Urobilinogen

*Recommended by Subdivision F (October 1982) Guidelines.

Results: There were no compound-related changes in any urinary parameters.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed; (F designates organs weighed after fixation in formalin):

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Peripheral nerve (sciatic nerve)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)
X	Stomach*	X	Lymph nodes*	X	Pituitary*
	Duodenum*	XX	Spleen*		
X	Jejunum*	X	Thymus*		
X	Ileum*	X	Eyes (optic nerve)*		
X	Cecum*		Urogenital		Glandular
X	Colon*	FXX	Kidneys*	FXX	Adrenals*
X	Rectum*	X	Urinary bladder*		Lacrimal gland
XX	Liver*	FXX	Testes*	X	Mammary gland*
X	Gallbladder*	X	Epididymides	FXX	Thyroids/ parathyroids*
X	Pancreas*	X	Prostate		Harderian glands
		X	Seminal vesicle		
	Respiratory	FXX	Ovaries		Other
X	Trachea*	XX	Uterus*	X	Bone (sternum)*
XX	Lung*	X	Vagina	X	Skeletal muscle*
X	Larynx/pharynx			X	Skin
				X	All gross lesions and masses

With the exception of one tissue mass, tissues from animals sacrificed at week 26 were not examined. Histopathologic examinations were performed on all animals that died or were sacrificed moribund or were sacrificed by design after 52, 56, and 96 weeks.

*Recommended by Subdivision F (October 1982) Guidelines.

Results:

a. Organ weights: There were no significant changes in organ weights or organ-to-body or organ-to-brain weight ratios in males after 26, 52 weeks or at the terminal sacrifice with the exception that the heart-to-body weight ratio was increased in high-dose males at 26 weeks. There were several significant ($p = 0.05$ or 0.01) increases in organ-to-body weight ratios in females receiving 1000 and 4000 pm. These changes were generally correlated with reductions of body weights and were not accompanied by increases in absolute organ weights or organ-to-brain weight ratios. Table 8 summarizes data for brain, kidney, and liver weights. Weight changes in heart, adrenal, and lungs were not consistent with time or dose.

b. Gross finding: There were no increases in the incidence of gross findings related to dosing.

c. Microscopic Pathology:

1) Nonneoplastic: Table 9 summarizes frequently occurring lesions in mice that died, were sacrificed moribund, or sacrificed by design after 52 or 95 weeks. Amyloidosis in several tissues showed statistically significant increases in dosed groups. When the number of mice from each group with amyloidosis at any site was compared there was no increase related to dosing. The incidence was fairly high as early a the 52-week sacrifice (62% of males and 20% of females in all groups combined). Incidence of amyloidosis is summarized in Table 10. Amyloidosis was not considered to be related to dosing with simazine.

2) Neoplastic: Table 11 summarizes neoplastic findings. There were no increases in dosed groups in any neoplasm.

TABLE 8. Mean Organ Weights (± S.E.) and Organ-to-Body Weight Ratios in Female Mice Fed Simazine Technical for 95 Weeks

	Dietary level (ppm)			
Organ/Interval	0	40	1000	4000
Brain				
Week 26 (g)	0.516 ± 0.008	0.512 ± 0.013	0.525 ± 0.008	0.499 ± 0.012
(% b.wt.)	1.70 ± 0.09	1.68 ± 0.05	1.98 ± 0.06	2.07 ± 0.04**
Week 52 (g)	0.493 ± 0.006	0.531 ± 0.017	0.536 ± 0.011	0.0525 ± 0.019
(% b.wt.)	1.50 ± 0.07	1.57 ± 0.6	1.84 ± 0.04**	1.93 ± 0.05**
Week 95 (g)	0.550 ± 0.009	0.535 ± 0.007	0.551 ± 0.013	0.510 ± 0.009*
(% b.wt.)	1.72 ± 0.06	1.70 ± 0.05	1.87 ± 0.06	2.009 ± 0.045**
Kidneys				
Week 26 (g)	0.431 ± 0.014	0.447 ± 0.010	0.429 ± 0.011	0.417 ± 0.020
(% b.wt.)	1.41 ± 0.05	1.46 ± 0.04	1.61 ± 0.04**	1.72 ± 0.05**
Week 52 (g)	0.500 ± 0.023	0.465 ± 0.007	0.454 ± 0.018	0.498 ± 0.030
(% b.wt.)	1.50 ± 0.05	1.38 ± 0.05	1.55 ± 0.06	1.82 ± 0.06**
Week 95 (g)	0.553 ± 0.033	0.554 ± 0.015	0.499 ± 0.010	0.425 ± 0.013**
(% b.wt.)	1.71 ± 0.10	1.75 ± 0.06	1.68 ± 0.04	1.66 ± 0.03
Liver				
Week 26 (g)	1.30 ± 0.05	1.31 ± 0.03	1.32 ± 0.06	1.24 ± 0.05
(% b.wt.)	4.22 ± 0.12	4.31 ± 0.12	4.91 ± 0.10**	5.14 ± 0.17**
Week 52 (g)	1.40 ± 0.07	1.38 ± 0.04	1.40 ± 0.05	1.46 ± 0.10
(% b.wt.)	4.19 ± 0.16	4.08 ± 0.13	4.78 ± 0.14*	5.29 ± 0.17**
Week 95 (g)	1.92 ± 0.18	1.55 ± 0.04	1.62 ± 0.05	1.62 ± 0.06**
(b.wt.)	5.90 ± 0.50	4.86 ± 0.14	5.45 ± 0.18	5.54 ± 0.13

*Significantly different from control value, p ≤0.05.
**Significantly different from control value, p ≤0.01.

TABLE 9. Nonneoplastic Findings Frequent in Mice Fed Simazine[a] Technical in the Diet for 95 Weeks

	Dose level (ppm)							
	Males				Females			
Organ/Findings	0	40	1000	4000	0	40	1000	4000
Adrenals	(68)[b]	(66)	(68)	(69)	(69)	(70)	(70)	(69)
Amyloid	33	36	42	34	15	18	14	21
Spindle cell hyperplasia	23	17	15	12	45	46	44	49
Bone marrow	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Myeloid hyperplasia	9	6	3	7	0	4	2	2
Heart	(71)	(70)	(70)	(71)	(70)	(70)	(69)	(70)
Amyloid	39	29	44	38	9	9	2	16*
Thrombosis	10	7	7	10	3	3	3	2
Intestine, small	(70)	(69)	(69)	(70)	(70)	(70)	(69)	(70)
Amyloid	47	46	51	42	32	30	29	22
Kidney	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Amyloid	44	47	48	39	25	27	19	19
Mononuclear cell foci	6	5	8	8	5	4	10	1
Liver	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Amyloid	29	28	40*	32	11	15	13	14
Lungs	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Amyloid	10	4	7	3	1	3	2	0
Histocytosis	5	3	3	1	6	10	5	3
Lymph node	(58)	(64)	(57)	(53)	(66)	(64)	(65)	(58)
Amyloid	19	19	19	22	5	8	10	12*
Hematopoiesis	7	1	3	12	5	2	2	0
Ovaries					(68)	(68)	(66)	(67)
Amyloid					18	16	10	13
Cyst(s)					22	26	18	18
Salivary glands	(71)	(70)	(70)	(71)	(70)	(69)	(68)	(71)
Amyloid	12	16	24**	13	2	6	4	8*
Spleen	(70)	(70)	(70)	(70)	(70)	(69)	(69)	(71)
Amyloid	7	14	11	11	4	11*	5	4
Hyperplasia	9	6	3	12	6	5	8	5

(continued)

Stomach								
Amyloid	8	6	14	6	5	4	2	2
Testes	(71)	(68)	(70)	(70)				
Amyloid	24	18	29	29				
Thyroid	(68)	(68)	(66)	(67)	(60)	(64)	(66)	(67)
Amyloid	22	26	28	24	7	15*	12	16*
Uterus					(70)	(70)	(70)	(70)
Amyloid					1	2	2	9**

[a]Does not include animals in the recovery group sacrificed after 56 weeks.
[b]The numbers in parentheses are the number of tissues examined histologically.
*Significantly different from control values at $p < 0.05$.
**Significantly different from control values at $p < 0.01$.

TABLE 10. Incidence of Mice with Amyloidosis in Simazine Feeding Study

Dose level (ppm)							
Males				Females			
0	40	1000	4000	0	40	1000	4000
52 Weeks							
8/11	6/10	7/10	5/11	2/10	0/10	4/10	2/11
95 Weeks							
48/60	49/60	52/60	45/60	37/60	34/60	28/60	28/60

TABLE 11. Neoplastic Findings in Mice Fed Simazine Technical for 94 Weeks

Organ/Neoplasm	Dietary level (ppm)							
	Males				Females			
	0	40	1000	4000	0	40	1000	4000
Eye	(70)[a]	(69)	(69)	(69)	(68)	(69)	(70)	(71)
Harderian carcinoma	0	0	0	0	0	0	1	1
Liver	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Hemangioma/hemangiosarcoma	0	1	0	1	0	0	0	0
Hepatocarcinoma	6	4	2	1	0	1	1	0
Hepatocellular adenoma	1	1	1	1	0	0	0	0
Lungs	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Adenocarcinoma	3	4	4	3	2	4	3	2
Adenoma	4	3	2	6	6	4	4	5
Ovary					(68)	(70)	68	70
Adenocarcinoma					0	0	0	1
Adenoma					0	1	1	1
Luteal cell tumor, benign					0	0	2	1
Luteal cell tumor, malignant					0	0	1	0
Pituitary	(54)	(55)	(53)	(51)	(57)	(57)	(57)	(59)
Adenocarcinoma	0	0	0	0	0	0	0	0
Adenoma	0	0	0	1	3	0	1	0
Stomach	(70)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Carcinoma	0	0	0	0	0	0	0	1
Systemic	(71)	(70)	(70)	(71)	(70)	(70)	(70)	(71)
Lymphoma, malignant	1	2	1	3	11	7	8	6
Leukemia	0	1	0	0	1	2	3	3
Histiocytic sarcoma	1	1	0	0	5	4	3	2
Testis	(71)	(68)	(70)	(71)				
Interstitial cell tumor	2	2	0	0				
Uterus					(70)	(70)	(70)	(70)
Adenocarcinoma					1	3	1	0
Adenoma					0	0	2	0
Endometrial stromal sarcoma					0	0	1	1
Hemangioma/hemangiosarcoma					2	4	2	1
Sarcoma (nonspecific)					0	1	0	0

[a]The values in parentheses are the number of tissues examined histologically; includes animal that died, were sacrificed moribund or were sacrificed by design after 52 and 95 weeks.

D. STUDY AUTHORS' CONCLUSIONS:

Under the conditions of the study, simazine technical was not oncogenic in CD-1 mice when administered in the feed at concentrations of 0, 40, 1000, or 4000 ppm for 95 weeks. Amyloidosis and/or intracardiac thrombosis were the major causes of death and moribundity. These lesions were considered incidental since they were found at approximately the same incidence in dosed and control mice. There was no evidence of a compound-related effect on survival or target organ toxicity. Reduced body weights, food and water consumption were found in mid- and high-dose groups. Erythroid parameters and organ weight alterations were found in the same groups. Based on reductions of 14 and 19 percent in body weight gain in males and females, the maximum tolerated dose (MTD) was considered to be 1000 ppm and the NOEL 40 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study protocol was acceptable for a chronic toxicity/oncogenicity study in mice. The conduct and reporting of the study were adequate. Sufficient blood was not available for measurement of all the clinical chemistry parameters. This is to be expected in a mouse study.

We assess that the decreased mean weight of mid- and high-dose males and females as well as a decrease in weight gain establish a maximum tolerated dose. The decrease in weight gain correlated with decreased food and water consumption. A decrease in mean body weights noted at four intervals in low-dose males probably indicates a threshold level for an effect. We agree with the study authors' assessment that the decreases were not of toxicologic importance; they were less than 4% of the body weight and there were no corresponding effects in females. The effects of dosing on hematology parameters were not severe and were of doubtful toxicologic importance. Organ weight changes in females were associated with decreased terminal body weights and their importance is doubtful in the absence of any gross or histological correlates.

The incidence of malignant lymphoma was higher in control females than in dosed groups. All values, however, were within the range of incidence found in other laboratories for this strain of mouse. The historical incidence in the testing laboratory was not provided.

We agree with the study authors conclusions that the NOEL was 40 ppm and that there was no oncogenic effect under the conditions of this study.

EPA Reviewer: Artensie R. Flowers, PhD. MPH **Signature:** _____
SIMB, Health Effects Division (7509C) **Date** _____
EPA Secondary Reviewer: _____ **Signature:** _____
[Insert Branch], Health Effects Division (7509C) **Date** _____

Template version 11/01

TXR#: 0050653

DATA EVALUATION RECORD - SUPPLEMENT
See TXR 007240 for original review

STUDY TYPE: Combined chronic toxicity/carcinogenicity diet - Rats; OPPTS 870.4300 [§83-5]; OECD 453.

PC CODE: 080807 **DP BARCODE:** D274214
SUBMISSION NO.: S562293

TEST MATERIAL (PURITY): Simazine Technical (96.9%)
SYNONYMS: NA

CITATION: McCormick, C.C., Arthur, A.T. and Green, J.D.(1988). Simazine-technical:104 week oral chronic toxicity and carcinogenicity study in rats. Pharmaceutical Div., Ciba-Geigy. Laboratory report number: 2-011-09. April 12, 1988. MRID:40614405. Unpublished.

Hart, S. (1993). Simazine Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats (Ovarian Re-evaluation). Ciba-Geigy Corp. Final Report: Lab Project Number: F-00181. MRID: 43029701. Unpublished..

SPONSOR: Ciba-Geigy Corporation, Greensboro, NC

EXECUTIVE SUMMARY:

In a combined chronic / carcinogenicity study (MRID 40614405, 43029701), simazine (96.9% a.i., Batch FL 850614) was administered via the diet to 680 Sprague-Dawley rats (Crl:VAF/Plus CD®): 40 rats/sex in the control and high dose groups of the chronic phase, 30 rat/sex in the low and intermediate dose groups of the chronic phase, and 50 rats/sex/dose in the carcinogenicity phase. Each group was exposed to the test material at dose levels of 0, 10 (LDT), 100(MDT), 1000 (HDT) ppm (0, 0.41/0.52, 4.2/5.3 and 45.8/63.1 mg/kg/day, males/females) for 52 weeks (chronic) or 104 weeks (carcinogenicity).

Over the duration of the study, significant decreases in body weight and body weight gain were noted in the HDT group for both sexes compared to control. At the end of the study body weight gain was decreased 27% for males and 28% for females. Food consumption in the HDT group was significantly decreased in both males and females throughout most of the study. Food consumption was decreased by 10-16% in males and 6-11% in females depending on the time

point.

Many hematology parameters in HDT females were statistically different from controls after 104 weeks and were as follows: decreased red blood cell counts (RBC); decreased hemoglobin (HGB) and hematocrit (HCT) values; increased mean corpuscular hemoglobin; increased mean corpuscular hemoglobin concentration (at day 174); increased white blood cell counts; increased platelet counts; increased percent neutrophils and decreased lymphocyte count. The MDT female group displayed statistically significant decreases in HCT and HGB at 104 weeks and significant increases in platelet counts at 104 weeks. In males, MCHC was significantly increased in the HDT group at 52 weeks. Leukocyte counts were significantly lower in both MDT and HDT males at 77 weeks.

Many clinical chemistry parameters were altered following exposure to simazine. The only alteration which is likely attributable to compound exposure, though, is the decrease in serum glucose levels seen in HDT female rats at 52, 77, and 104 weeks. In HDT females, heart wts. were increased 23%, kidney wts. were increased 31% and liver wts. were increased 430%. In HDT males liver and testes wts., relative to body weight, were significantly increased (20% for liver, 28% testes). Heart weight was decreased absolutely by 15% and was decreased relative to brain wt. by 13%.

In the original study (MRID 40614405), no nonneoplastic findings in the male rat were significantly increased in any dose group vs controls. In females, liver hematopoiesis, splenic hematopoiesis and cystic mammary glandular hyperplasia were all significantly increased in incidence in the HDT vs the controls. A reevaluation of selected ovarian tissue and Sertoli cell changes (MRID 43029701) subsequent to the original study determined that there was a significantly increase after 24 months exposure to 1000 ppm in-ovarian atrophy and Sertoll Cell hyperplasia. Animals treated for 52 weeks and then allowed 52 weeks recovery did not display these changes.

Mortality in the entire study (both chronic and oncogenicity portions up to completion of study) was increased HDT females. Mortality was 80% in these animals compared to 66% in the control females. Male HDT mortality dropped from 61% for controls to 40% for HDT.

Increases in mammary, pituitary and kidney neoplasms were seen in HDT females. Only the increases in mammary tumors were found to be statistically significant. Mammary carcinomas ($p<0.001$, 35/70 HDT vs 14/70 control) and fibroadenomas ($p<0.01$, 40/70 vs 22/70) were significantly increased in HDT females, while only mammary carcinomas were increased ($p<0.05$) in mid-dose females. Males displayed non-significant increases in liver tumors (combined adenoma and carcinoma 1/70 control vs 6/70 HDT).

The LOEL is 100 ppm (4.2/5.3 mg/kg/day, males/females) based on decreases in HCT, HGB and platelet counts in females and decreased leukocyte counts in males. The NOEL is 10 ppm (0.41/0.52 mg/kg/day, males/females).

At the doses tested, there was a treatment related increase in mammary carcinomas and fibroadenomas tumor incidence when compared to controls. Dosing was considered adequate based on hematology alterations,

This chronic/carcinogenicity study in the rat is **Acceptable-Guideline** and satisfies the guideline requirement for a chronic/ carcinogenicity study [(OPPTS 870.4300); OECD 453] in rat.

COMMENTS: This is a revised Executive Summary only and does not alter the conclusions of the previous review.

DER #1

Chemical Name:Simazine
2-Year Feeding/Carcinogenicity Study in Rats
Sponsor Name: Ciba-Geigy
Year of Study: 1988
MRID No: 40614405
HED Doc. No. TXR 007240

CITATION: McCormick, C.C., Arthur, A.T. and Green, J.D.(1988) Simazine-technical: 104 week oral chronic toxicity and carcinogenicty study in rats. Pharmaceutical Div., Ciba-Geigy. Laboratory report number: 2-011-09.April 12, 1988. MRID:40614405. Unpublished.

EXECUTIVE SUMMARY:

In a combined chronic toxicity/carcinogenicity study (MRID 40614405), Simazine, 96.9% a.i., was administered to 680 Sprague-Dawley rats. Fifty rats/sex/dose (400 of 680 rats) were used for the carcinogenicity portion of this study. The rats in the carcinogenicity portion were exposed to test article for 104 weeks. The other 280 rats were used in the chronic portion of the study. The chronic portion of the study was divided into three sections; 1. an interim sacrifice at 52 weeks at which 10 animals sex/dose were sacrificed; 2. a 104 week exposed section in which 20 animals sex/dose were exposed and sacrificed; 3. and a section which was only at the control and high dose in which 10 animals sex/dose were exposed for 52 weeks and then allowed 52 weeks of recovery. Test article was admixed in the diet at dose levels of 0, 10 (LDT), 100 (MDT), 1000 (HDT)ppm (0, 0.41/0.52, 4.2/5.3 and 63.1/45.8 mg/kg/day males/females.

HDT body weight and body weight gains for both sexes was significantly decreased compared to controls throughout the study. Male body wt. was 22% lower and per-cent body wt. gain was decreased 27% at 104 weeks. Female body wt. was decreased 23% while per-cent body wt. change was 28% less. Food consumption in the HDT group was significantly decreased in both males and females throughout the study although at week 104 food consumption between HDT and controls was only slightly decreased. Food consumption was decreased by 10-16% in males and 6-11% in females depending on the timepoint.

Many hematology parameters appeared to be altered by simazine treatment in the females. Parameters which were statistically significantly altered and the weeks at which they were altered in the female HDT *vs* controls are: decreased red blood cell counts (RBC)(25, 52, 65, 77 and 104 weeks); decreased hemoglobin (HGB) and hematocrit (HCT)values (52, 77 and 104); increased mean corpuscular hemoglobin (52, 77 and 104); increased mean corpuscular hemoglobin concentration (MCHC)(25); increased white blood cell counts (25, 52, 65, 77 and 104); increased per cent neutrophils (52)decreased lymphocyte count(52 weeks). Alkaline phosphatase activity was increased at 52 and 77 weeks in females who were exposed for 52 weeks then allowed 52 weeks recovery. The 100 ppm group (mid dose tested - MDT) displayed statistically significant decreases in HCT and HGB at 104 weeks and significant increases in platelet counts at 104 weeks.

Male hematology parameters were not altered to near the extent that the females were. MCHC was significantly increased in HDT males at 52 weeks. Leukocyte counts were significantly lower in both MDT and HDT males at 77 weeks.

Many clinical chemistry parameters were altered following

exposure to simazine. The only alteration which is likely attributable to compound exposure, though, is the decrease in serum glucose levels seen in HDT female rats at 52, 77, and 104 weeks.
In HDT females, heart wts. were increased 23%, kidney wts. were increased 31% and liver wts. were increased 43%. In HDT males liver and testes wts., relative to body weight, were significantly increased(20% for liver, 28% testes). Heart weight was decreased absolutely by 15% and was decreased relative to brain wt. by 13%.
In the original study (MRID 40614405), no nonneoplastic findings in the male rat were significantly increased in any dose group *vs* controls. In females, liver hematopoiesis, splenic hematopoiesis and cystic mammary glandular hyperplasia were all significantly increased in incidence in the HDT *vs* the controls. A reevaluation of selected ovarian tissue and Sertoli cell changes (MRID 43029701) subsequent to the original DER determined that there was a significantly increase after 24 months exposure to 1000 ppm in ovarian atrophy and Sertoli Cell hyperplasia. Animals treated for 52 weeks and then allowed 52 weeks recovery did not display these changes.
Mortality in the entire study (both chronic and oncogenicity portions up to completion of study) was increased HDT females. Mortality was 80% in these animals compared to 66% in the control females. Male HDT mortality dropped from 61% for controls to 40% for HDT.
Increases in mammary, pituitary and kidney neoplasms were seen in HDT females. Only the increases in mammary tumors were found to be statistically significant. Mammary carcinomas ($p<0.001$, 35/70 HDT *vs* 14/70 control)and fibroadenomas($p<0.01$, 40/70 *vs* 22/70) were significantly increased in both MDT and HDT females while carcinomas were increased ($p<0.05$) in mid-dose females. Males displayed non-significant increases in liver tumors (combined adenoma and carcinoma 1/70 control *vs* 6/70 HDT).
The LOEL is 100 ppm (4.2/5.3 mg/kg/day, males/females) based on decreases in HCT, HGB and platelet counts in females and decreased leukocyte counts in males. The NOEL is 10 ppm (0.41/0.52 mg/kg/day, males/females).

At the doses tested, there was a treatment related increase in mammary carcinomas and fibroadenomas tumor incidence when compared to controls. Dosing was considered adequate based on hematology alterations.

This chronic toxicity/carcinogenicity study in the rat is **Acceptable-Guideline**, and satisfies the guideline requirement for a combined chronic toxicity/carcinogenicity study (83-5) in the rat.

Author: Robert Haus 6/12/98

Attachment # 9 83-5

Reviewed By: Y.M. Ioannou 10/28/88
Section II, Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: M. Copley 11/4/88
Section II, Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

Study Type: Chronic Toxicity/Carcinogenicity (Rat) (83-5)

TOX Chem No.: 740
MRID No.: 406144-05

Test Material: Simazine Technical

Study No(s).: 2-011-09

Sponsor: Ciba-Geigy Corporation, Greensboro, NC

Testing Facility: Ciba-Geigy Corporation
Pharmaceuticals Division
Summit, NJ

Title of Report: Simazine-Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats.

Author(s): C.C. McCormick, A.T. Arthur, J.D. Green

Report Issued: April 12, 1988

Conclusions:

The LEL for chronic toxicity of Simazine Technical in Sprague-Dawley rats was found to be 100 ppm (5.3 mg/kg/day) based on depression of body weight gains and depression of values for the hematology parameters, RBC, HGB and HCT in female rats. The NOEL was found to be 10 ppm (0.5 mg/kg/day).

Simazine Technical was found to be oncogenic in female rats, inducing mammary tumors at dose levels of 100 ppm (5.3 mg/kg/day) and 1000 ppm (63.1 mg/kg/day).

In male rats Simazine appears to induce the formation of liver tumors (hepatocellular adenomas/carcinomas) at the dose level of 1000 ppm (45.8 mg/kg/day).

Classification: Core-Minimum

Materials and Methods:

Male and female Sprague-Dawley rats [Crl:VAF/Plus™ CD®. (SD)Br] obtained from Charles River, Kingston, NY, approximately 6 weeks old and weighing 126 to 189 g (males) or 101 to 167 g (females) were used throughout this study. Upon arrival all animals were examined for their health status and only healthy animals were included in the study. Ophthalmoscopic examinations were performed on all animals and necropsy and serologic determinations were performed on five males and five females, randomly selected. The rats were acclimated to laboratory conditions for approximately 3 weeks and after the first week of acclimation they were housed in individual cages, identified with Monel ear tags and provided with food (Ground Purina Certified Rodent Chow #5002) and tap water ad libitum. Animal cages were kept in a room where the temperature was maintained at 73 ± 5 °F, the relative humidity at 50 ± 20 percent, with a 12-hour light/dark cycle.

Study Design:

A total of 340 male and 340 female rats were used in this study. The rats were randomly divided into four major groups/sex and exposed to dietary concentrations of Simazine Technical as shown on the following page (abstracted from the original report).

For the preparation of the test diets, Simazine Technical (Batch FL 850614) with a purity of 96.9 percent (personal communication with Tom Parshley of Ciba-Geigy) was mixed with powdered Certified Purina Rodent Chow #5002, at intervals based on the stability of the test article admixtures at room temperature. This stability was reportedly at least 21 days for the low-dose (10 ppm) and at least 40 days for the mid- and high-dose admixtures. Test article concentrations in the diet were determined at study initiation and at approximately 4-week intervals thereafter for the first year, and at 8-week intervals for the second year on study. The homogeneity of Simazine in diet admixtures was determined twice (study week 1 and 63) during the study.

All animals were observed daily for clinical symptoms of toxicity and mortality. Body weights were recorded on weeks -3 and -2, weekly during weeks 1 through 13, biweekly during weeks 14 through 25 and monthly thereafter for the remainder of the study. Food consumption was determined weekly for weeks 1 through 13, biweekly for weeks 14 through 25 and monthly thereafter. Water consumption was measured on weeks 1, 2, 53 through 64, and 102 on study. All animals were palpated for masses at 4-week intervals for the first 9 months on study and at 2-week intervals thereafter.

Treatment Schedule:

The test article/feed admixtures were available ad libitum at concentrations of 10, 100 or 1000 ppm. The control group received untreated Certified Purina Rodent Chow #5002 ad libitum. Chronic phase animals consisted of 40 rats/sex in the control and high-dose groups and 30 rats/sex in the low- and intermediate-dose groups, while carcinogenicity phase animals consisted of 50 rats/sex/group. The test article/ feed admixtures were administered 7 days/week for a minimum of 104 consecutive weeks according to the following schedule:

Group	Phase	Number of Rats		Dietary Concentration (ppm)	Least Number of Dose Weeks
		Male	Female		
1	Chronic[a]	10	10	0	52
		10	10		52 + 52-wk recovery
		20	20		104
	Carcinogenicity[b]	50	50		104
2	Chronic[a]	10	10	10	52
		20	20		104
	Carcinogenicity[b]	50	50		104
3	Chronic[a]	10	10	100	52
		20	20		104
	Carcinogenicity[b]	50	50		104
4	Chronic[a]	10	10	1000	52
		10	10		52 + 52-wk recovery
		20	20		104
	Carcinogenicity[b]	50	50		104

[a]After approximately 52 weeks of treatment, 10 rats/sex/group from the chronic phase were sacrificed and an additional 10 rats/sex also from the chronic phase in the control and high-dose groups were maintained on untreated (control) diet for approximately 52 weeks at which time the remaining animals were sacrificed. After approximately 104 weeks of treatment, the remaining animals from the chronic phase were sacrificed.

[b]After approximately 104 weeks of treatment, the remaining animals from the carcinogenicity phase were sacrificed.

Ophthalmoscopic evaluations were carried out on weeks -2, 25, 52, 72 through 76, and 104 and for recovery animals on week 65 on study. Blood smears for animals sacrificed moribund during the study were evaluated for differential count and red cell morphology.

For hematology and clinical chemistry determinations blood was collected from the right orbital sinus of male and female rats lightly anesthetized with ether. For urinalysis, urine samples were collected during a 16-hour overnight period from nonfasted animals for volume determinations while freshly voided urine was used for determination of all other urinalysis parameters. Hematology, clinical chemistry and urinalysis determinations were carried out based on the following schedule (abstracted from the original report):

				No. Rats Used for Clinical Lab. Determinations[a]					
	No. Rats		Week of	Hematology[b]		Biochem.[b]		Urinalysis[b]	
Group	M	F	Sac.	M	F	M	F	M	F
Baseline[c]	20	20	-1	10	10	10	10	10	10
1	10	10	105-106	10	10	10	10	10	10
	20	20	105-106	10	10	10	10	10	10
2	20	20	105-106	10	10	10	10	10	10
3	20	20	105-106	10	10	10	10	10	10
4	10	10	105-106	10	10	10	10	10	10
	20	20	105-106	10	10	10	10	10	10

[a]Animals from the carcinogenicity phase were used for these determinations at the final sampling period in order to have 10/sex/group.
[b]Analyses were conducted predose (test week -1) on baseline animals, at weeks 25 and 26, 77 and 78, and 104 on animals assigned to the 104-weeks chronic phase, and weeks 52, 65 and 66, 78 and 104 on animals assigned to the recovery phase.
[c]Baseline animals included 10/sex for hematology 10/sex for biochemistry and urinalysis. These data have been maintained in the raw data file for the study.

For hematology, clinical chemistry, and urinalysis the following CHECKED (X) paramaters were examined:

1. Hematology

X		X	
X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count*	X	Mean corpuscular volume (MCV)
		X	Reticulocytes

2. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase*	X	A/G ratio
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma GT		

3. Urinalysis

X		X	
	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

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*Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 level)
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*		Urogenital		Glandular
X	Cecum*	XX	Kidneys*	XX	Adrenals*
X	Colon*	X	Urinary bladder*		Lacrimal gland
X	Rectum*	XX	Testes*	X	Mammary gland*
XX	Liver*	XX	Epididymides	X	Parathyroids*
	Gallbladder*	X	Prostate	X	Thyroids*
X	Pancreas*	X	Seminal vesicle		Other
	Respiratory	XX	Ovaries	X	Bone*
X	Trachea*	X	Uterus	X	Skeletal muscle*
X	Lung*			X	Skin
				X	All gross lesions and masses

*Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

Histopathological examinations were conducted on all gross lesions involving tissue masses. In addition, formalin-fixed pituitary tissue was processed so that, if needed, sections could be stained using immunocytochemical staining procedures for the identification of prolactin.

Statistical Evaluation:

(Abstracted from the original report - see Appendix A)

Results:

Chemical analyses of feed admixtures established that a) Simazine was stable in the diet (at room temperature) for at least 21 days for the low-dose (10 ppm) and for at least 40 days for the mid- and high-dose levels. (The authors did not give any justification as to why low-dose admixtures were tested for stability for only 21 days); b) Simazine concentrations in the diet were in close agreement with the target concentrations of 10, 100, and 1000 ppm; and c) Simazine homogeneity in diet admixtures was at an acceptable level as evidenced by the almost identical values obtained from samples within the same dose level.

Clinical Signs - Although a great variety of clinical signs were observed throughout the study, the incidence and/or frequency of these signs was for the most part comparable between the Simazine-treated and the control groups. Clinical signs that were of higher

incidence in the high-dose groups compared to controls included: Tissue mass in females (12 versus 38 for control and HDT, respectively); swollen appendages in males (7 versus 17 for control and HDT, respectively); alopecia/ general hairloss in females (2 versus 5 for control and HDT, respectively).

Mortality - As illustrated below, the mortality rate for the interim sacrifice and terminal sacrifice (main study) groups was very low during weeks 0 through 52 on study. However, a high rate of mortality was reported for the main study between weeks 53 and 106 (terminal sacrifice). For males the survival rate in the HDT was significantly higher than the control group (39 versus 60% for the control and HDT, respectively); on the contrary, in females the survival rate was in general lower than the males for all groups; the survival rate for the HDT females (20%) was much lower than the controls (34%).

Study	Week of Study	Sex	Mortality Ratio 0 ppm	10 ppm	100 ppm	1000 ppm
Interim	0-52	M	0/10 (0)[1]	1/10 (10)	0/10 (0)	0/10 (0)
		F	0/10 (0)	0/10 (0)	1/10 (10)	1/10 (10)
Main	0-52	M	2/70 (3)	0/70 (0)	0/70 (0)	0/10 (0)
		F	0/70 (0)	2/70 (10)	7/70 (10)	4/70 (6)
	53-106	M	41/68 (60)	46/70 (66)	39/70 (56)	28/70 (40)
		F	46/70 (66)	45/68 (66)	46/63 (73)	52/66 (79)
	0-106	M	43/70 (61)	46/70 (66)	39/70 (56)	28/70 (40)
		F	46/70 (66)	47/70 (67)	53/70 (76)	56/70 (80)

[1]Number in parentheses denotes percent mortality.

Palpable Masses - The incidence of palpable masses (confirmed at necropsy) was significantly higher in females of the HDT (1000 ppm) compared to the controls. For the controls 37/90 (41%) animals had palpable masses while for the high-dose group 60/80 (75%) animals had palpable masses. For the low- and mid-dose groups palpable masses were of approximately the same incidence as the controls. In males the incidence of palpable masses was comparable in all groups.

Ophthalmological Examinations - None of the ocular effects observed could be attributed to the test article since the incidence and frequency of these effects were comparable between treated and control groups.

Body Weight - Data presented here indicate that mean body weights for male and female rats of the HDT (1000 ppm) were statistically significantly lower than the control group beginning on day 7 on study and continuing to study termination (day 728) Table 1.

For female rats of the mid-dose group (100 ppm) statistically significantly lower mean body weights as compared to controls were observed at different time intervals throughout the study and at study termination. Mean body weight gains were also statistically significantly lower in male and female rats of the high-dose groups as compared to controls throughout the study. For male and female animals of the mid-dose groups (100 ppm) statistically significantly lower body weight gains were seen occasionally at different time intervals but not at study termination.

Food Consumption - A statistically significant reduction in food consumption was observed in male rats of the HDT (1000 ppm) beginning at day 7 (first time point measured) and continuing until day 700 on study (4 weeks before sacrifice), Table 2. Statistically significant depression of food intake was also reported for female rats of the HDT on days 7 through 560 on study, but not during the final 6 months on study (Table 2). The reduced food consumption in males and females of the HDT correlated with the lower body weight and body weight gains in the same groups throughout the study. In rats of the low- and mid-dose groups (males and females) change in food consumption was seen only rarely during the study.

Based on the food consumption and the animal body weight (at mid-period) the authors calculated the following mean daily dose intake in mg/kg for each treatment group for both sexes:

Sex	Group	Dietary Concentration (ppm)	Dietary Concentration mg/kg/day	Mean Daily Dose mg/kg/day	Range mg/kg/day
M	2	10	0.5	0.41	0.27 - 1.29
	3	100	5.0	4.17	2.75 - 13.12
	4	1000	50.0	45.77	37.48 - 119.40
F	2	10	0.5	0.52	0.30 - 1.36
	3	100	5.0	5.34	3.27 - 14.50
	4	1000	50.0	63.10	50.04 - 125.24

These results indicate that females were receiving mean daily doses, on a mg/kg basis, between 27 and 38 percent higher than the corresponding male dose groups. The range for mean daily doses was for the most part comparable between the two sexes.

Table 1
Mean Body Weights and Percent Body Weight Gains at Selected Time Intervals

	Sex	Day on Study	Dose (ppm) 0	10	100	1000
Mean body weight (g)	M	0	160.4 (1.3)[1]	161.3 (1.3)	160.4 (1.3)	158.8 (1.3)
		7	206.9 (1.6)	207.3 (1.9)	204.9 (1.6)	188.4** (1.5)
		98	542.9 (5.7)	538.3 (5.8)	529.6 (4.7)	434.7** (4.1)
		364	757.5 (10.7)	774.6 (9.9)	731.2 (9.0)	573.7** (7.0)
		532	795.5 (16.7)	835.1 (14.6)	782.4 (11.5)	592.1** (8.4)
		728	744.8 (29.3)	785.2 (31.9)	744.2 (18.2)	582.5** (10.5)
Mean body weight gain (%)	M	7	29.0 (0.3)	28.6 (0.9)	27.8* (0.3)	18.7** (0.3)
		98	239.1 (3.2)	234.3 (3.3)	231.1 (2.9)	174.2** (2.2)
		364	374.0 (6.4)	381.2 (5.7)	357.3 (5.7)	261.7** (3.9)
		532	399.4 (10.3)	417.6 (9.0)	392.1 (8.0)	277.3** (5.1)
		728	372.8 (19.8)	389.9 (20.8)	368.5 (13.0)	270.6** (6.2)
Body Weight Gain Change Compared to Controls (%)	M	7	-	-1.4	-4.1	-35.5
		98	-	-2.0	-3.3	-27.1
		364	-	+1.9	-4.5	-30.0
		532	-	+4.6	-1.8	-30.6
		728	-	+4.6	-1.2	-27.4

[1] Numbers in parentheses denote standard error.

*,** Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Table 1 (cont'd)

	Sex	Day on Study	Dose (ppm) 0	10	100	1000
Mean body weight (g)	F	0	133.6 (1.1)[1]	135.4 (1.2)	126.0 (1.8)	131.1 (1.0)
		7	156.8 (1.2)	157.2 (1.4)	150.1** (1.7)	143.7** (1.1)
		98	303.6 (3.1)	298.2 (3.7)	295.4 (4.2)	239.6** (2.3)
		364	451.0 (7.4)	451.7 (7.9)	424.7* (8.2)	321.3** (4.1)
		532	524.3 (13.0)	502.6 (12.1)	497.5 (14.2)	362.2** (9.2)
		728	570.2 (26.3)	543.3 (22.2)	473.0* (31.4)	440.2** (24.4)
Mean body weight gain (%)	F	7	17.4 (0.4)	16.2 (0.4)	19.6* (0.7)	9.6** (0.4)
		98	127.6 (1.9)	120.5* (1.9)	135.7* (2.8)	82.7** (1.2)
		364	237.9 (5.0)	233.8 (4.8)	240.0 (6.0)	146.1** (2.7)
		532	296.0 (10.2)	274.1 (8.9)	307.3 (11.3)	178.6** (6.7)
		728	331.3 (18.7)	314.0 (19.4)	301.3 (27.8)	238.2* (18.1)
Body Weight Gain Change Compared to Controls (%)	F	7	-	-6.9	+12.6	-44.8
		98	-	-5.6	+ 6.3	-35.2
		364	-	-1.7	+ 0.9	-38.6
		532	-	-7.4	+ 3.8	-39.7
		728	-	-5.2	- 9.1	-28.1

[1]Numbers in paretheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Table 2
Mean Food Consumption at Selected Time Intervals

Day on Study	Food Consumption (Grams/Week) Dose (ppm) Males 0	10	100	1000	Females 0	10	100	1000
7	141.5 (1.2)[1]	142.6 (2.1)	143.8 (1.3)	124.4** (1.3)	114.3 (1.4)	119.5* (1.3)	120.1* (2.1)	103.3** (1.7)
98	182.9 (2.1)	173.9** (2.3)	188.6 (2.2)	154.4** (2.0)	132.8 (1.7)	133.5 (1.9)	141.5** (2.1)	122.2** (1.7)
364	177.1 (2.5)	180.8 (2.7)	174.3 (2.5)	160.2** (1.6)	145.4 (2.5)	156.7* (2.6)	142.2 (2.4)	137.0* (1.6)
532	188.9 (3.3)	178.5 (4.4)	184.5 (3.2)	164.2** (2.7)	149.4 (3.0)	136.1 (4.9)	143.3 (4.9)	132.4* (4.2)
728	158.7 (5.9)	148.2 (7.8)	146.9 (6.0)	155.0 (5.1)	126.9 (6.2)	116.3 (7.1)	110.0 (10.5)	151.8 (11.1)

[1] Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Water Consumption - Some differences in water consumption were seen between the treated and the control groups. These differences are not, however, considered toxicologically important due to their random ocurrence and the lack of a dose-response.

Hematology - As shown in Table 3, a number of hematology parameters appeared to be affected by Simazine treatment. This apparent treatment-related effect was pronounced mainly in the high-dose group females (1000 ppm) at most time points of sampling. Statistically significant changes between the control and high-dose group values were seen in females in the following parameters: Red blood cell (RBC) count-depressed at all time points; hemoglobin (HGB)-depressed on days 361, 537, and 725 on study; hematocrit (HCT)-depressed on days 361, 537, and 725 of sampling; mean corpuscular hemoglobin (MCHB)elevated on days 361, 537, and 725 of sampling; mean corpuscular hemoglobin concentration (MCHC)-elevated on day 174 of sampling; white blood cell count (WBC)-elevated on days 174, 361, 537, and 725 of sampling; neutrophils (percent)-elevated on day 361 of sampling; and lymphocytes-depressed on day 361 of sampling. Changes in these parameters, although only occasionally statistically significant, were also observed in the mid-dose group females (Table 3). Comparable changes between the control and the high-dose group were also seen in females of the recovery group.

In males, the MCHC was statistically significantly higher in the HDT compared to the control group on day 361 of sampling (with an apparent dose-related trend); the leukocyte count was statistically significantly lower than controls in the mid- and high-dose groups on day 537 of sampling. Other changes seen were not considered treatment-related. In males of the recovery group hematology parameter values were comparable for the most part between the HDT and the control groups. Statistically significantly lower values were seen on day 537 for mean corpuscular volume (MCV) and on days 537 and 725 for MCHB.

Clinical Chemistry - A number of clinical chemistry parameters were found to be statistically significantly different between treated and control groups at different time intervals in both sexes. However, it appears that the only changes on clinical chemistry parameters that could possibly be attributed to Simazine treatment were the depression of glucose levels in female rats at all time points of sampling (Table 4). Glucose depression was also seen with the recovery group females at all time points tested except on day 725.

Table 3
Effect of Simazine on Selected Hematology Parameters - Female Rats

Parameter	Day of Test	Dose (ppm)					
		Main Study				Recovery Group	
		0	10	100	1000	0	1000
RBC (x10 E6/Cmm)	174	7.1 (0.2)[1]	6.7 (0.2)	7.0 (0.3)	6.8 (0.1)		
	361	6.3 (0.1)	6.2 (0.1)	6.1 (0.1)	5.4** (0.2)	6.4 (0.1)	5.8* (0.2)
	537	6.9 (0.1)	6.8 (0.1)	6.8 (0.2)	5.8** (0.2)		
	725	6.4 (0.3)	6.6 (0.1)	5.6 (0.3)	5.0** (0.3)		
HGB (gm/dL)	361	14.1 (0.2)	14.2 (0.1)	14.2 (0.2)	12.7** (0.4)	14.3 (0.1)	13.3** (0.3)
	537	14.8 (0.2)	14.6 (0.2)	14.7 (0.3)	13.2** (0.2)	14.8 (0.2)	13.8* (0.3)
	725	14.5 (0.5)	14.5 (0.2)	12.7* (0.5)	12.3** (0.5)		
HCT (%)	361	41.4 (0.6)	41.2 (0.5)	41.5 (0.9)	36.1** (1.3)	42.6 (0.5)	38.2** (0.9)
	537	43.5 (0.5)	42.6 (0.5)	42.8 (1.5)	37.9** (0.9)	44.0 (0.6)	41.2* (0.9)
	725	41.4 (1.4)	41.2 (0.7)	36.4* (1.4)	34.3** (1.5)		
MCHB (mmicro gm)	361	22.3 (0.3)	22.7 (0.3)	23.3 (0.3)	23.7* (0.4)		
	537	21.3 (0.2)	21.6 (0.4)	21.8 (0.2)	22.8** (0.4)		
	725	22.6 (0.5)	22.0 (0.3)	22.8 (6.5)	24.6* (0.5)		
MCHC (%)	174	34.4 (0.2)	34.0 (0.3)	34.0 (0.3)	35.5* (0.3)		
WBC (x10 E3/Cmm)	361	6.3 (0.5)	6.6 (0.5)	8.2 (1.0)	8.6* (0.6)	6.7 (0.5)	7.8 (0.6
	725	7.8 (1.1)	7.4 (0.7)	10.2 (1.3)	14.0** (1.7)		

[1]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Table 3 (cont'd)
Effect of Simazine on Selected Hematology Parameters - Female Rats

Parameter	Day of Test	Dose (ppm) Main Study 0	10	100	1000	Recovery Group 0	1000
Platelet (x10E3/Cmm)	174	865.2 (55.2)[1]	1003.8 (53.5)	947.8 (43.5)	1140.4** (40.1)		
	361	871.7 (41.6)	888.2 (35.3)	945.4 (60.8)	1062.0* (32.0)		
	537	880.0 (49.0)	970.0 (37.8)	1014.9 (56.2)	1212.3** (44.5)		
	725	980.4 (64.2)	952.9 (45.0)	1224.9* (85.7)	1189.0 (46.1)		
Neutrophils (%)	361	16.3 (1.5)	21.1 (2.8)	22.2 (3.2)	33.6** (3.7)		
Lymphocytes (%)	361	78.7 (1.6)	72.9 (2.9)	69.5 (3.7)	61.9** (3.4)		

[1]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Also, alkaline phosphatase activity was elevated at all time points measured reaching statistical significance on days 361 and 455 of sampling, in females of the recovery group (note: for the recovery group only parameters of the control and high-dose group were measured). For the same group (recovery group, females) the activities of SGOT and SGPT were also depressed slightly throughout the study.

Urinalysis - Most of the urinalysis parameters measured were found to be comparable in the control and treated groups in both sexes. Statistically significantly higher urine volume was obtained on day 358 of analysis in the females of the HDT, and the males and females of the recovery group. Urine specific gravity was statistically significantly decreased on days 358 and 454 of analysis in females of the recovery group.

Organ Weights

a. Absolute Organ Weights - A statistically significant decrease in absolute organ weight was observed as follows: Brain, high dose males at the 52-week sacrifice; heart, high dose males at the terminal sacrifice; and liver, high dose females at the 52-week sacrifice (Table 5).

Table 4
Effect of Simazine on Selected Clinical Chemistry Parameters

Parameter	Day of Test	Dose (ppm) Males 0	Males 10	Males 100	Males 1000	Females 0	Females 10	Females 100	Females 1000
Glucose (mg/dL)	174					176.4 (4.4)	179.3 (6.4)	176.7 (9.1)	144.8** (4.7)[1]
	361					143.9 (4.0)	143.8 (4.8)	140.4 (5.0)	125.0* (4.0)
	537					148.0 (9.0)	147.9 (4.2)	134.6 (6.5)	127.1 (4.2)
	725	141.4 (8.1)	100.2** (9.7)	130.4 (6.3)	157.2 (5.6)	143.3 (7.4)	131.7 (7.0)	114.5* (10.3)	118.5 (4.2)
Cholesterol (mg/dL)	537					108.7 (8.7)	140.1 (14.6)	154.9* (10.5)	135.2 (7.7)
Total Bilir. (mg/dL)	361	0.38 (0.03)	0.34 (0.03)	0.25** (0.02)	0.25** (0.02)	0.46 (0.07)	0.51 (0.11)	0.35 (0.03)	0.22** (0.02)
	537	0.44 (0.08)	0.41 (0.05)	0.32 (0.05)	0.30 (0.02)	0.48 (0.11)	0.36 (0.08)	0.21* (0.02)	0.26 (0.04)
Albumin (gm/dL)	174	3.5 (0.04)	3.5 (0.04)	3.5 (0.05)	3.6* (0.04)				
	361	3.6 (0.09)	3.6 (0.07)	3.8 (0.07)	3.9* (0.04)				
Globulin (gm/dL)	174					2.4 (0.1)	2.4 (0.1)	2.5 (0.1)	2.7* (0.1)
	361	3.0 (0.09)	3.0 (0.11)	2.7* (0.10)	2.9 (0.07)				
Album./Globul.	174					1.9	1.8	1.8	1.7*
	725					1.4	1.4	1.4	1.2
Calcium (mg/dL)	361	10.21 (0.09)	9.99 (0.08)	9.90** (0.03)	9.95* (0.05)				
Sodium (meq/L)	725					142.5 (1.0)	144.1 (0.5)	145.1 (0.8)	145.7* (0.5)

[1]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

b. Relative Organ Weights - In male rats of the high-dose group the relative weight for brain, liver and testes was statistically significantly higher than controls at 52 weeks and 104 weeks of sacrifice. In females, the relative weight of brain, heart, adrenal, kidney, liver, and ovaries at the interim sacrifice (52 weeks) was statistically significantly higher than controls in the high-dose group. For kidneys, statistical significance was also seen with the mid-dose group. Additionally, the relative weight of heart, kidney, and liver was statistically significantly higher than controls in the high-dose group at terminal sacrifice (104 weeks) (Table 5).

c. Organ-to-Brain Weight Ratios - Statistically significantly lower organ-to-brain weight ratios were observed for the heart of the high dose group males at the 104-week sacrifice and for the liver of the high-dose group females at the 52-week sacrifice (Table 5).

Table 5
Effect of Simazine on Organ Weights

Organ	Dose (ppm) 0		10		100		1000	
	52 Weeks[1]	104 Weeks	52 Weeks	104 Weeks	52 Weeks	104 Weeks	52 Weeks	104 Week
Males								
Brain - Absolute (g)	2.29 (0.05)[2]		2.31 (0.03)		2.30 (0.03)		2.16* (0.03)	
- % of Bodyweight	0.32 (0.01)	0.35 (0.02)	0.30 (0.01)	0.33 (0.01)	0.31 (0.01)	0.34 (0.01)	0.37* (0.02)	0.4 (0.0
Heart - Absolute (g)		2.15 (0.07)		2.15 (0.08)		2.08 (0.07)		1.8 (0.0
- % of Brain		93.04 (3.02)		91.49 (3.44)		87.45 (2.55)		81.1 (1.7
Liver - % of Bodyweight	3.11 (0.14)	2.56 (0.13)	2.95 (0.08)	2.41 (0.12)	3.00 (0.07)	2.53 (0.10)	3.50* (0.11)	3.0 (0.0
Testes - % of Bodyweight	0.74 (0.03)	0.67 (0.05)	0.64 (0.03)	0.61 (0.04)	0.66 (0.04)	0.64 (0.03)	0.86* (0.04)	0.8 (0.0
Females								
Brain - % of Bodyweight	0.42 (0.02)		0.43 (0.03)		0.50 (0.02)		0.64** (0.02)	
Heart - % of Bodyweight	0.24 (0.01)	0.30 (0.02)	0.25 (0.01)	0.30 (0.02)	0.27 (0.01)	0.32 (0.01)	0.33** (0.01)	0.3 (0.0
Adrenal - % of Bodyweight	0.015 (0.001)		0.016 (0.001)		0.015 (0.001)		0.022** (0.001)	
Kidney - % of Bodyweight	0.54 (0.02)	0.65 (0.05)	0.58 (0.03)	0.65 (0.04)	0.63* (0.02)	0.68 (0.04)	0.77** (0.02)	0.8 (0.
Liver - Absolute (g)	15.10 (0.84)		15.94 (0.73)		13.43 (0.76)		12.42* (0.72)	
- % of Brain	741.4 (34.8)		759.4 (40.7)		649.1 (32.7)		606.2* (36.4)	
- % of Bodyweight	3.08 (0.10)	2.32 (0.11)	3.17 (0.13)	2.35 (0.11)	3.18 (0.12)	2.54 (0.12)	3.81** (0.11)	3.3 (0.(
Ovary - % of Bodyweight	0.021 (0.001)		0.022 (0.002)		0.022 (0.003)		0.030* (0.004)	

[1]Interim sacrifice.
[2]Numbers in parentheses denote standard error.
*,**Statistically significantly different from controls; $p < 0.05$ and $p < 0.01$, respectively.

Gross Pathology - Gross pathology, performed on all animals that died during the study, sacrificed at moribund condition or sacrificed at the scheduled study period (52 weeks or 104 weeks on study) revealed that the incidence of macroscopic lesions in the simazine treated groups was not statistically significantly different from that of the control groups in either sex. Numerical differences in the incidence of gross lesions were seen in some instances, especially in the high-dose groups, and are reported in Table 6 for the record.

Histopathological Lesions - Histopathological examination revealed numerous nonneoplastic and neoplastic lesions in many tissues of male and female rats.

Male Rats - Nonneoplastic Lesions - As shown in Table 7, the incidence of a number of noneoplastic lesions in male rats was comparable between the controls and the low- and mid-dose groups tested, but slightly higher than controls in the high-dose group.

Neoplastic Lesions - Although not statistically significant, the incidence of several neoplastic lesions in male rats was numerically higher than controls mainly in the high dose group. Table 7 shows that these neoplastic lesions involved: adrenal-cortical adenoma; kidney-adenoma and carcinoma; liver-adenoma and carcinoma; and thyroid-C-cell adenoma and carcinoma.

Female Rats - Nonneoplastic Lesions - The incidence of several nonneoplastic lesions in female rats was statistically significantly higher in the high dose group compared to controls as follows: mammary gland-cystic glandular hyperplasia; liver-hematopoiesis; and spleen-hematopoiesis (Table 8). Other nonneoplastic lesions were found to be only numerically higher than controls mainly in the high-dose group as shown in Table 8.

Neoplastic Lesions - The incidence of mammary gland carcinomas in female rats was found to be statistically significantly higher than controls in the mid- and high-dose groups as shown in Table 8. The incidence of mammary gland fibroadenomas was also statistically significantly higher than controls in the high-dose group. Mammary gland adenomas were only numerically higher than controls in the low- and high-dose groups. The incidence of pituitary adenomas was extremely high in all groups including controls (Table 8). Pituitary carcinomas were of higher incidence in the low- and high-dose groups compared to controls. Although the incidence of kidney tubular adenomas was only 2/70 in the high-dose group (and 0/70 in the other groups), because of its rarity in Sprague-Dawley rats this tumor appears in Table 8 for the record.

Table 6
Summary of Macroscopical Observations

Macroscopical Observation	Dose (ppm)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Main Study (104 Weeks)								
Kidney - distended	2/70[1]	2/70	0/70	3/70	2/70	3/70	2/70	9/70
Ovary - cyst					2/70	2/70	2/70	4/70
Pituitary - enlarged	23/70	26/70	29/70	20/70	52/70	48/70	47/70	62/70
Postappendage - tissue mass					5/70	1/70	2/70	12/70
Skin (chest and thorax) - tissue mass	1/70	1/70	3/70	7/70	14/70	18/70	9/70	40/70
Skin (inguinal) - tissue mass					23/70	22/70	22/70	37/70
Spleen - enlarged					2/70	2/70	1/70	9/70
Interim Sacrifice (52 Weeks)								
Pituitary - enlarged					1/10	3/10	3/10	4/10
Skin (inguinal) - tissue mass					0/10	0/10	1/10	4/10
Recovery Group (104 Weeks)								
Skin (chest and thorax) - tissue mass					1/10			5/10

[1]Number of rats with specified observation/total number of tissues examined.

Table 7
Summary of Histopathological Lesions - Male Rats

Histopathological Observation[1]	Dose (ppm) 0	10	100	1000
Neoplastic Lesions				
Adrenal - cortical adenoma	0/69[2]	0/70	1/69	2/69
Kidney - Adenoma	0/70	0/70	0/70	1/70
- Carcinoma (primary)	0/70	0/70	0/70	2/70
Liver - Hepatocellular adenoma	1/70	1/70	0/70	3/70
- Hepatocarcinoma	0/70	2/70	4/70	3/70
- Combined adenoma and/or carcinoma	1/70	3/70	4/70	6/70
Thyroid - C-cell adenoma	2/70	7/69	5/69	6/70
- C-cell carcinoma	2/70	1/69	1/69	3/70
- Combined adenoma and/or carcinoma	4/70	8/69	6/69	9/70
Pituitary - Adenoma	42/69	47/70	47/70	38/70
Nonneoplastic Lesions				
Adrenal - Cortical hypertrophy/ cystic degeneration	7/69	4/70	6/69	13/69
- Focal cortical hyperplasia	2/69	2/70	3/69	7/69
Liver - Hyperplasia	2/70	0/70	0/70	0/70
Pituitary - Hyperplasia	12/69	14/70	10/70	15/70
Skin - Chronic lymphocytic inflammation	1/70	0/68	1/69	5/70
Testis - Focal interstitial cell hyperplasia	6/70	2/70	8/70	11/70
Thyroid - Focal interstitial cell hyperplasia	7/70	3/69	5/69	9/70

[1]Main study only (interim sacrifice and recovery groups not included).
[2]Number of rats with specified observation/total number of tissues examined.

Table 8
Summary of Histopathological Lesions - Female Rats

Histopathological Observations[1]	Dose (ppm) 0	10	100	1000
Neoplastic Lesions				
Mammary - Adenoma	2/70[2]	4/70	1/70	5/70
- Carcinoma	14/70	13/70	19/70*	35/70***
- Fibroadenoma	22/70	27/70	19/70	40/70**
Pituitary - Adenoma	62/70	57/70	60/70	57/70
- Carcinoma	1/70	3/70	0/70	6/70
Kidney - Adenoma (tubular)	0/70	0/70	0/70	2/70
Nonneoplastic Lesions				
Mammary - Cystic glandular hyperplasia	51/70	50/70	53/70	65/70***
Pituitary - Hyperplasia	2/70	6/70	3/69	2/70
Kidney - Hydronephrosis	3/70	0/70	0/70	6/70
- Epithelial hyperplasia pelvic	0/70	0/70	0/70	3/70
Adrenal - Focal medul. hyperplasia	0/70	4/70	3/70	3/70
Liver - Hematopoiesis	0/70	1/70	1/70	5/70*
Spleen - Hematopoiesis	3/70	1/70	1/70	10/70*
Thyroid - Focal interstitial cell hyperplasia	0/70	2/70	2/70	4/70

[1]Main study only (interim sacrifice and recovery groups not included).
[2]Number of rats with specified observation/total number of tissues examined.
*,**,***Indicates significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Discussion:

The present study has investigated the chronic toxicity and oncogenic potential of simazine in male and female Sprague-Dawley rats. The selection of the dose levels used in this study (10, 100 and 1000 ppm) was based on the results of a 90-day feeding study in rats whereby the dose levels of 2000 and 4000 ppm resulted in significant body weight depression (20-40% compared to controls) while the low dose of 200 ppm was established as the NOEL (personal communication with Mr. Tom Parshley of Ciba-Geigy).

Analytical data presented by the authors indicate that: simazine concentrations in the diet were approximately the same as target concentrations (of 10, 100, or 1000 ppm); the test article was homogeneously distributed in the diet (for all dose levels); and the test article was stable in the diet for at least 40 days for the mid- and high-dose levels and 21 days for the low-dose level.

The clinical signs were approximately of equal incidence between the control and the simazine-treated groups. The occasional higher incidence of some clinical signs that was seen with the HDT was not considered to be treatment-related due to the lack of dose-response and/or the fact that this higher incidence did not persist throughout the study. Female animals of the high-dose group had a higher incidence of palpable masses, reflecting the higher incidence of tumors found in this group, as compared to controls.

Mortality data presented here indicate that mortality rates in female rats were very high in all groups (control and treated) with the MDT and HDT resulting in slightly higher mortality than the control group. Mortality rates in male rats were reported to be slightly lower than controls for the MDT and HDT. Further statistical analysis of the mortality rates in both sexes (conducted by C.J. Nelson, Science Analysis and Coordination Branch, Health Effects Division) has shown that in female rats mortality was statistically significantly higher in the mid- and high-dose groups with a statistically significant increasing trend; in male rats, mortality was statistically significantly decreased in the HDT compared to the control group with a statistically significant decreasing trend as shown below.

Dose	Mortality	
	Male	Female
0	48/80** (60)	53/80** (66)
10	47/71 (66)	47/70 (67)
100	39/70 (56)	53/71** (75)
1000	28/70** (40)	57/71** (80)

() Denotes percent.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose; ** $p < 0.01$

These findings might suggest a sex-related difference in susceptibility to the test article possibly resulting from the higher incidence of life-threatening tumors in female than in male rats.

Mean body weights and mean body weight gains for male and female rats of the high-dose groups, were statistically significantly lower than controls throughout the study. Terminal mean body weights were 22 and 23 percent lower than controls in males and females of the HDT, respectively, while mean body weight gains (at study termination) were depressed by 27 and 28 percent in males and females, respectively. In females of the MDT there was a 17 percent decrease in mean body weights and 9 percent body weight gain decrement at study termination. No effect was seen in males of the MDT. However, according to the authors, female animals received 28-38 percent higher concentrations of the test article throughout the study. This finding might partly explain the higher toxicity observed in females of the MDT. The lower mean body weights and body weight gains correlated with the statistically significantly lower food consumption for male and female rats of the HDT compared to controls. These results suggest that the lower body weight gains could be attributed, at least to some extent to the lower food intake possibly due to the unpalatability of the test article in the diet. However, a closer look at food consumtion- grams of food consumed per kg body weight- indicated that food intake for male and female rats of the HDT was significantly higher than the other groups, ranging from 5.7 percent (at day 98) to 25 percent (at day 728) for males and from 17 percent (at day 98) to 55 percent (at day 728) for females, suggesting that food efficiency for animals of the HDT was very low compared to the other groups.

Hematology data indicate that treatment of female rats with Simazine at 1000 ppm results in anemic animals as indicated by the simultaneous statistically significant decrease in RBC, HGB, and HCT at different time points of sampling. We request, however, that the authors provide the Agency with the appropriate bone marrow determinations (Myeloid/Erythroid ratio) for further evaluation of this effect (see Appendix B). Other parameters that appeared to be affected by the high dose of the test article included the statistically significant increase in WBC, MCHC, MCHB, and platelets and neutrophils, indicating in general an abnormal state in these animals. No major changes in these parameters between the treated and control groups were reported in male rats.

From the clinical chemistry parameters measured only the changes seen in glucose values in the females of the HDT appeared to be treatment-related. The lower glucose values, however, might be the indirect result of depressed body weights in this group.

Although the absolute and/or relative weights of a number of organs were statistically significantly different between the control and treated groups, such differences do not appear to be

of major toxicological significance since changes in organ weights, in general, were not associated with concomitant clinical chemistry changes and/or changes in pathological lesions (macroscopic and/or microscopic) in the same organs which could explain these organ weight changes.

The following points can made concerning the oncogenic potential of simazine in male and female Sprague-Dawley rats:

1. Female Rats

a. Mammary Gland - In female rats of the main study there was a statistically significant increase in the incidence of mammary carcinomas in the mid- and high-dose groups compared to controls. A statistically significantly higher incidence of fibroadenomas was seen in the high-dose group. When the incidence of these lesions was calculated separately for female animals that died (or sacrificed moribund) or animals that survived to terminal sacrifice, the following incidence of mammary tumors was seen.

	Lesion	Dose (ppm) 0	10	100	1000
Early deaths (prior to terminal sacrifice)	Adenoma[1]	2/46[1]	3/47	1/53	4/56
	Carcinoma	10/46	9/47	12/53	28/56**
	Fibroadenoma	14/46	17/47	11/53	28/56**
Scheduled sacrifice (104 weeks)	Adenoma	0/24	1/23	0/17	1/14
	Carcinoma	4/24	4/23	7/17	7/14*
	Fibroadenoma	8/24	10/23	8/17	12/14*
Combined incidence	Adenoma	2/70	4/70	1/70	5/70
	Carcinoma	14/70	13/70	19/70*	35/70**
	Fibroadenoma	22/70	27/70	19/70	40/70**

[1]Number of animals with specified observation/total number of tissues examined.
*,**,*** Indicates significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

As the statistical analyses carried out by the authors for different tumors in male and female rats were determined to be inadequate, further statistical evaluation for the major tumors listed in Tables 7 and 8, was conducted by C.J. Nelson, Statistician, Science Analysis and Coordination Branch, Health Effects Division. Data presented in all tables below are the combined tumor incidence from the 52-week interim sacrifice and the 104-week study. The incidence of mammary tumors in female rats is presented in the following table.

Simazine Sprague-Dawley Rat Study--Female Mammary Gland Tumor Rates+ and Peto Prevalence Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma Fibroadenoma	23/89 (26)	20/78a (26)	11/71 (15)	21/75 (28)
	p = 0.0689	p = 0.302	p = 0.177	p = 0.123
Carcinoma	16/89 (18)	13/80 (16)	20/75b (27)	40/78 (51)
	p < 0.0001**	p = 0.4740	p = 0.0392*	p < 0.000
Adenoma Carcinoma	39/89 (44)	33/80 (41)	31/75 (41)	61/78 (78)
	p < 0.0001**	p = 0.4064	p = 0.2229	p < 0.000

a First adenoma observed at 48 weeks in dose 10 ppm and the first fibroadenoma observed at 52 weeks in dose 0, 10, and 1000 ppm.
b First carcinoma observed at 48 weeks in dose 100 ppm.
+ Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
() Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes p < 0.05 and ** denotes p < 0.01

These results indicated that there was a statistically significant dose-related trend in mammary carcinomas and in combined adenomas and carcinomas. The incidence of mammary carcinomas was statistically significantly increased in the mid- and high-dose groups compared to controls; also the incidence of combined adenomas and carcinomas was significantly higher in the HDT compared to controls. Mammary carcinomas (in the main study - 104-week sacrifice) contributed, according to the authors, to the increased mortality in the high-dose group animals (1000 ppm). A higher incidence of mammary carcinomas was also seen in the recovery study (52 weeks treatment with 1000 ppm followed by 52 weeks of recovery) 1/10 vs. 4/10, for the control and HDT, respectively.

In female rats the incidence of hyperplastic changes (cystic glandular hyperplasia) in the mammary gland was statistically significantly higher than controls

in the HDT. This finding corroborates the observed high incidence of tumors in the HDT. It is generally understood that the higher tumor incidence correlates directly with a higher incidence of hyperplastic changes.

c. Pituitary Gland - In female rats the incidence of pituitary (pars distalis) carcinoma was found to be higher than controls in the HDT. The authors reported that this incidence was statistically significant when the Peto life table method of analysis was used. The incidence of adenomas was found to be extremely high in all groups but the authors reported that the incidence in the mid- and high-dose groups was statistically significantly increased when Peto's method was used for analysis (when contribution to death is considered). Further statistical analysis of these tumors (total tumor analysis) indicated, as shown below, that the incidence of combined adenomas/carcinomas in the mid- and high-dose groups was statistically significantly higher than controls with a significant dose-related trend.

Simazine Sprague-Dawley Rat Study--Female Pituitary Gland Tumor Rates+, Fatal Tumor Analysis and Generalized K/W Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	73/89 (82.0)	57/80 (71.2)	63/77[a] (81.8)	61/79 (77.2)
	p = 0.0033**	p = 0.9944	p = 0.0206*	p = 0.0030**
Carcinoma	1/73 (1.4)	3/61 (4.9)	0/52 (0.0)	6/53[b] (11.3)
	p = 0.0010**	p = 0.2351	p = 0.4545	p = 0.0153*
Adenoma Carcinoma	74/89 (83.1)	60/80 (75.0)	63/77 (81.8)	67/79 (84.8)
	p = 0.0005**	p = 0.8351	p = 0.0251*	p = 0.0005**

+ Number of tumor bearing animals/Number of animals at risk (excluding animals not examined).
() Percent
[a] First adenoma observed at 35 weeks in dose 100 ppm
[b] First carcinoma observed at 72 weeks in dose 1000 ppm.
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

The authors reported that these tumors (adenomas and carcinomas) were considered to be fatal "by virtue of their size and compression of the mid-brain," and thus contributed to the decreased survivability of the mid- and high-dose group females. Although these tumors (adenomas/carcinomas) were of approximately the same numerical incidence in all groups (treated and control) examination of the Kaplan-Meier survival curves (constructed by C.J. Nelson, Statistician, SACB/HED) indicates that the onset of these tumors is 4 to 15 weeks earlier in the mid- and high-dose groups as compared to the control and low dose groups.

For further evaluation of these tumor data the authors are requested to provide the Agency with historical control data as shown in Appendix B. Furthermore, the authors should provide the Agency with the results of the immunocytochemical staining of the pituitary for identification of prolactin (see Appendix B).

c. Kidney - Based on Peto's time-adjusted trend analysis the incidence of kidney tubular adenoma in female rats of the high-dose group was statistically significantly higher than controls. Additional analysis of these data (see below) indicated that there was a statistically significant dose-related trend for the incidence of this tumor. This tumor is considered to be very rare with a spontaneous

Simazine Sprague-Dawley Rat Study--Female Kidney Tubule Tumor Rates+, Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	0/74 (0.0)	0/62 (0.0)	0/54 (0.0)	2/55[c] (3.6)
	p = 0.0042**	p = 1.0000	p = 1.0000	p = 0.1799

[c]First adenoma observed at 71 weeks in dose 1000 ppm. No carcinomas were coded.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
() Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

incidence of 0 to 1 percent in this strain of rats, as compared to 3.6 percent incidence in the high-dose group in this study. This finding does not appear to be of major biological significance. The sponsor is however requested to provide the Agency with historical control data for this tumor, as shown in Appendix B.

2. Male Rats

a. Liver - In male rats the incidence of hepatocellular adenomas or carcinomas was very low in all treated and control groups (0-5%). As shown in the table below, the incidence of combined adenomas and carcinomas was statistically significantly higher in the high dose group compared to controls possibly suggesting oncogenic potential of simazine to male rats.

Simazine Sprague-Dawley Rat Study--Male Liver Tumor Rates+, Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	1/88 (1.1)	2/79[a] (2.5)	0/80 (0.0)	3/80 (3.8)
	p = 0.0824	p = 0.4594	p = 0.5238	p = 0.275
Carcinoma	0/88 (0.0)	2/79 (2.5)	4/80[b] (5.0)	3/80 (3.8)
	p = 0.2169	p = 0.2223	p = 0.0494*	p = 0.105
Adenoma Carcinoma	1/88 (1.1)	4/79 (5.1)	4/80 (5.0)	6/80 (7.5)
	p = 0.0643	p = 0.1519	p = 0.1554	p = 0.044

[a]First adenoma observed at 52 weeks in dose 10 ppm.
[b]First carcinoma observed at 99 weeks in dose 100 ppm.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before 52 weeks or animals not examined).
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

The incidence of hyperplastic changes, however, was very low in the control (2/70) and nonexistent in the treated groups (0/70, Table 7).

b. Thyroid - Although the incidence of combined thyroid C-cell adenomas and carcinomas was numerically higher in all treated groups as compared to controls, as shown below there was no significant dose-related trend or statistical significance between treated and control groups. The incidence of hyperplastic changes was comparable to the incidence of tumors for each group.

Simazine Sprague-Dawley Rat Study--Male Thyroid C-cell Tumor Rates+, and Peto Prevalence Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	2/52 (4)	7/52[a] (13)	5/51 (10)	6/58 (10)
	p = 0.3355	p = 0.0606	p = 0.1082	p = 0.087(
Carcinoma	2/34 (6)	1/31 (3)	1/36 (3)	3/45[b] (7)
	p = 0.1762	p = 0.1082	p = 0.2881*	p = 0.418:
Adenoma Carcinoma	4/52 (8)	8/52 (15)	6/51 (12)	9/58 (16)
	p = 0.1924	p = 0.1965	p = 0.2261	p = 0.150!

[a]First adenoma observed at 89 weeks in dose 10 ppm.
[b]First carcinoma observed at 102 weeks in dose 1000 ppm.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
() Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

c. Kidney - As shown below a very low incidence of tubular adenomas and carcinomas was seen in male rats. A statistically significant dose-related trend was observed for the incidence of carcinomas as well as the incidence of combined adenomas and carcinomas. As in female rats, the very low incidence of this rare tumor in male rats does not appear to be of biological significance.

Simazine Sprague-Dawley Rat Study--Male Kidney Tubule Tumor Rates+ and Peto Prevalence Test Results

Dose (ppm)	0.000	10.000	100.000	1000.000
Adenoma	0/51 (0)	0/46 (0)	0/48 (0)	1/57[a] (2)
	p = 0.0543	p = 1.0000	p = 1.0000	p = 0.5278[b]
Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	2/65[c] (3)
	p = 0.0332*	p = 0.1660	p = 0.1821	p = 0.2091
Adenoma Carcinoma	1/66 (2)	0/62 (0)	0/64 (0)	3/65 (5)
	p = 0.0056**	p = 0.1410	p = 0.1721	p = 0.1087

[a]First adenoma observed at 92 weeks in dose 1000 ppm.
[b]The p values for adenomas were calculated using the Cochran-Armitage Trend Test and Fisher's Exact Test, since the Peto Prevalence method collapsed to one interval.
[c]First carcinoma observed at 78 weeks in dose 1000 ppm.
+Number of tumor-bearing animals/Number of animals at risk (excluding animals that died before the observation of the first tumor or animals not examined).
() Percent
Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.
* denotes $p < 0.05$ and ** denotes $p < 0.01$

Based on the aforementioned evaluation of the data we conclude that Simazine Technical is oncogenic in female Sprague-Dawley rats inducing the formation of mammary gland carcinomas. Simazine Technical also appears to increase the induction of liver tumors in male rats. We thus consider this chemical a candidate for Peer Review.

Conclusions:

The LEL for the chronic toxicity of Simazine Technical in Sprague-Dawley rats was found to be 100 ppm (5.3 mg/kg/day) for females (depression of body weight gains and depression of values for the hematology parameters, RBC, HGB and HCT). In males the LEL was found to be 1000 ppm (45.8 mg/kg/day) based on depression of body weight gains. The NOEL was 10 ppm (0.5 mg/kg/day) for females and 100 ppm (4.2 mg/kg/day) for males.

Simazine Technical was found to be oncogenic in female Sprague-Dawley rats inducing mammary tumors at dose levels of 100 ppm (5.3 mg/kg/day) and 1000 ppm (63.1 mg/kg/day).

In male rats Simazine appears to induce the formation of liver tumors at the dose level of 1000 ppm (45.8 mg/kg/day).

Classification: Core-Minimum

APPENDIX A

STATISTICAL EVALUATION:

Body Weight, Food and Water Consumption, Clinical Laboratory and Organ Weight Data: All numerical data that were generated in the course of the study were stored in the Beckman TOXSYS® data base in the IBM mainframe computer and maintained by Research Computing Services in the SEF. Individual animal data reports were generated by programs in the Beckman TOXSYS® system or programs developed by Research Computing Services. Statistical analyses were performed separately for each sex using the Statistical Analysis System (SAS) Version 5 and SUGI Supplemental Library, 1983 Edition on the IBM mainframe computer.

Tests for outliers and Bartlett's test for homogeneity of variances were performed to check deviations from the normal theory model. If the model assumptions were met, Dunnett's tests were performed to compare each of the treated groups versus the control. If significant model deviations were detected (either outliers were present or heterogenous variances were evident), supplemental analyses, including the use of appropriate data transformations, nonparametric tests or other multiple comparison procedures without assuming equal variances, were performed as needed. Descriptions of specific methods employed and additional references were added in the summary tables when supplemental analyses were performed. Nonparametric tests based on ranks were conducted on parameters that were known not to be normally distributed. A detailed description of the statistical methodology used in this study is presented in Section 6.

Pathology: All microscopic data were recorded by the pathologist or designee into the NO3 Pathology Data system in the Ardsley IBM mainframe computer. The data were tabulated by the appropriate pathology data system and if sample sizes were adequate, these data were analyzed separately for each sex by Fisher's exact tests. Incidences of lesions and their statistical significance were taken from each of the NO3-generated printouts (stored in the Archives of Toxicology/Pathology in the SEF building) and summarized in Appendix 9.6.1. In addition, tumor incidences were analyzed by a time adjusted analysis based on Peto's method. A detailed description of the statistical methodology used in this study can be found in Section 9.6.

Mortality: The days on test were regarded as censoring times for animals sacrificed on schedule and as true death times for animals that died or were sacrificed moribund. The survival distribution for each group and each sex was determined using Kaplan-Meier estimates. Nonparametric rank tests: Mantel-Cox logrank test for equality and test for linear trend were performed separately for each sex to test for differences between the survival curves of the treatment groups. If significant differences were found, follow-up pairwise comparisons based on these procedures were then performed to compare each treated group versus the control. A detailed description of the statistical methodology used in this study is given in Section 9.2.

APPENDIX B

Additional data are requested from the sponsor as follows:

1. Historical control data. Data obtained from Sprague-Dawley rats for the last five (5) years at Ciba-Geigy Laboratories (Summit, New Jersey) as follows:

   Mammary gland - adenomas, carcinomas and fibro-adenomas for female rats.

   Pituitary gland - adenomas and carcinomas for female rats.

   Kidney - tubular adenomas and carcinomas for male and female rats.

   Adrenal - cortical adenomas for male rats.

   Liver - adenomas and carcinomas for male rats.

   Thyroid - C-cell adenomas and carcinomas for male rats.

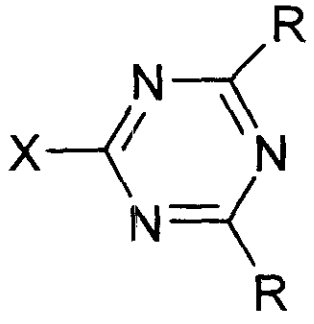
2. All available data on the immunocytochemical staining of the pituitary gland for identification of prolactin.

3. Bone marrow determinations for establishing the Myeloid/Erythroid ratio in all dose groups, males and females.

4. Provide justification for the selection of the dose levels used in this study.

5. Specify the purity of Simazine Technical used in the study.

The Grouping of a Series of Triazine Pesticides Based on a Common Mechanism of Toxicity



U.S. EPA Office of Pesticide Programs
Health Effects Division
March 2002

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ACRONYMS

CNS	Central Nervous System
DEA	Desethyl-s-atrazine
DIA	Desisopropyl-s-atrazine
DACT	Diaminochlorotriazine
F344	Fischer 344
FQPA	Food Quality Protection Act
FSH	Follicle-stimulating Hormone
GD	Gestation Days
GnRH	Gonadotropin Releasing Hormone
HA	2-Hydroxyatrazine
HLZ	Holtzman
HPG	Hypothalamic-pituitary-gonadal
LE	Long Evans
LH	Luteinizing Hormone
LOAEL	Lowest Observed Adverse Effect Level
NE	Norepinephrine
NOAEL	No Observed Adverse Effect Level
OPP	Office of Pesticide Programs
PND	Postnatal Days
SAP	Scientific Advisory Panel
SD	Sprague Dawley
US EPA	United States Environmental Protection Agency
WOE	Weight-of-Evidence

Executive Summary

This document discusses the available scientific evidence for determining whether a common mechanism of toxicity exists among certain triazine-containing pesticides. The weight-of-evidence (WOE) analysis used is similar to the general approach outlined in the January 29, 1999 ***Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity*** (http://www.epa.gov/oppfead1/trac/science/ #common and http://www.epa.gov/fedrgstr/EPA-PEST/1999/February/Day-05/6055.pdf). The group of triazine-containing chemicals considered as candidates for grouping in this document consists of the following pesticides: **atrazine, simazine, propazine, tribenuron-methyl (Express)**, and the degradants **2-hydroxyatrazine, desethyl-s-atrazine (DEA), desisopropyl-s-atrazine (DIA)**, and **diaminochlorotriazine (DACT)**.

Treatment of laboratory animals with these chemicals results in toxic effects such as mammary gland tumors in only female rats, attenuation of the lutenizing hormone (LH) surge, alteration of the estrous cycle, altered pregnancy maintenance, and delayed pubertal development. The development of mammary gland tumors in female rats is postulated to be associated with disruption of the hypothalamic-pituitary-gonadal (HPG) axis. In summary, the proposed mode of action for induction of mammary gland tumors in female rats by atrazine involves altered secretory activity of the HPG axis, beginning with a decrease in the release of gonadotropin releasing hormone (GnRH) by the hypothalamus followed by a consequent attenuation of the afternoon LH surge during the estrous cycle. As a result, ovulation does not occur and the estrous cycle is prolonged, thereby increasing the exposure to estrogen. Increased estrogen also stimulates prolactin secretion from the pituitary. The resultant endocrine milieu of enhanced or unopposed estrogen and prolactin secretion provides an environment that is conducive to the development of mammary gland tumors. Likewise, attenuation of the surge in LH, alteration of the estrous cycle, altered pregnancy maintenance, and delayed pubertal development are considered to be either manifestations or direct consequence of disruption of the HPG axis. This proposed mode of action of atrazine for reproductive developmental effects in female SD rats (considered in this document for grouping by a common mechanism of action) was presented by the Agency to the FIFRA Scientific Advisory Panel (SAP) in June 27-29, 2000 and found to be plausible.

Based on the available WOE, only **atrazine, simazine, propazine,** and the degradants **DEA, DIA** and **DACT** can be grouped by a common mechanism of toxicity for disruption of the hypothalamic-pituitary-gonadal (HPG) axis. Although some of the evidence may support including **Express** and/or **2-hydroxyatrazine**, the overall weight-of-evidence does not support their inclusion in the common mechanism group. If additional data become available to directly support their inclusion in the common mechanism group, these data would be considered.

Thus, in the absence of additional evidence that may support an alternative grouping, **atrazine, simazine, propazine,** and the degradants **DEA, DIA** and **DACT** will be considered as a common mechanism group for purposes of a cumulative risk assessment and as part of the tolerance reassessment process for triazine pesticides.

The Grouping of a Series of Triazine Pesticides Based on a Common Mechanism of Toxicity

I. Introduction

A. Background

The Food Quality Protection Act (FQPA) amended the laws under which EPA evaluates the safety of pesticide residues in food. Among other types of information EPA is to weigh when making safety decisions, the amendments direct EPA to consider "available information concerning the cumulative effects of such residues and other substances that have a common mechanism of toxicity." Sec. 408(b)(2)(D)(v) of the Federal Food Drug and Cosmetic Act. FQPA also directs EPA to apply the new safety standard to tolerances established prior to the passage of FQPA. Further, in carrying out the tolerance reassessment provisions of FQPA, EPA "shall give priority to review of the tolerances or exemptions that appear to pose the greatest risk to public health." Sec. 408(q)(2).

B. Purpose

The purpose of this document is to evaluate whether there is a common mechanism for the triazine pesticides or between the triazine pesticides and other pesticides or metabolites containing a s-triazine ring. OPP used a weight-of-evidence (WOE) approach that considered all pertinent information to determine whether triazine pesticides act via a common mechanism of toxicity. A stepwise process is outlined in the 1999 Guidance for Identifying Pesticide Chemicals and Other Substances That Have A Common Mechanism of Toxicity (http://www.epa.gov/oppfead1/trac/science/#common). The process starts with an initial grouping of chemicals based on having shared structural, toxicological and/or pesticidal properties (US EPA, 1999a). In a second phase, the steps that define the mechanism of toxicity for one or more chemicals in the group are identified. Finally, structural, toxicological and pharmacokinetic/pharmacodynamic data for the remaining chemicals in the group are examined to determine by WOE which of these possess the same mechanism of toxicity as the other compound(s) in the group. All those chemicals found to share the same mechanism of toxicity for a common toxic effect are considered to have been grouped by a common mechanism of toxicity.

It should be noted that since the passage of the FQPA, the term "mechanism of toxicity" has taken on a specific meaning in Agency-wide guidance documents. In the draft EPA guidelines for carcinogen risk assessment, the term "mode of action" is contrasted with "mechanism" which implies a more detailed molecular description of events than is meant by mode of action (US EPA, 1999b). The definition of "mechanism of toxicity", as implemented under FQPA, and thus used in OPP's common mechanism guidance (US EPA, 1999a), is equivalent to the definition of the term "mode of

action." Thus, "mechanism of toxicity" in this document is defined as "*the major steps leading to an adverse health effect following interaction of a pesticide with biological targets. All steps leading to an effect do not need to be specifically understood. Rather, it is the identification of the crucial events following chemical interaction (with biological targets) that are required in order to describe a mechanism of toxicity.*"

II. The Candidate Group of Pesticides

A. The Triazines

The term "the triazines" has traditionally been used by EPA to refer to a group of 3 pesticides, atrazine, simazine, and cyanazine. See "Atrazine, Simazine and Cyanazine; Notice of Initiation of Special Review," 59 FR 60412 (November 23, 1994). OPP labeled a slightly larger group of pesticides as 1,3,5-triazines in the schedule for tolerance reassessment. In addition to atrazine, simazine, and cyanazine, pesticides so labeled included propazine, ametryn, cyromazine and prometryn. Additionally, several other pesticides or pesticide metabolites contain a s-triazine ring.

The triazines, the 1,3,5- triazines, and other pesticides or metabolites containing a s-triazine ring are derivatives of the s-triazine moiety, manufactured by the reaction of trichlorocyanuric acid (Figure 1) with appropriate intermediates. Two of the three chlorine atoms on cyanuric acid are reactive and easily replaced with other groups to yield a variety of herbicidally active compounds. The third chlorine atom may remain (e.g., in position 2) or be replaced with a methylthio or methoxy group.

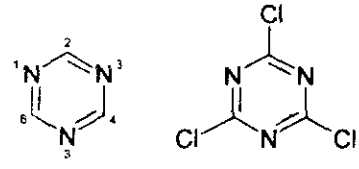


Figure 1. s-Triazine Ring (left) illustrating the ring-numbering convention and 2,4,6-trichlorocyanuric acid (right).

These compounds can be subdivided into several classes, depending on the side chain substitution: the s-triazine herbicides, the sulfonyl urea herbicides with an s-triazine moiety, and miscellaneous s-triazine herbicides. As depicted in Table 1, the s-triazine herbicides may be further subdivided by the presence of a chlorine, a methylthio, or a methoxy group in position 2 of the ring. In particular, the chloro-s-triazines comprise atrazine, simazine, propazine, terbutylazine, and cyanazine. This latter compound differs from the other 2-chloro-s-triazines by the presence of a cyano (CN) group. The methylthio s-triazines comprise ametryn, prometryn and terbutryn. The methoxy-s-triazines include prometon and terbumeton.

As depicted in Table 2, the sulfonyl urea herbicides with an s-triazine moiety comprise metsulfuron methyl, trisulfuron, chlorsulfuron, tribenuron methyl (Express), and DPX-M6316 (Harmony). These compounds differ from the s-triazine herbicides in having a bulky sulfonylurea group attached to carbon 2 of the triazine ring and in possessing an s-triazine ring with only one amino group

attached to it.

Other s-triazine pesticidal compounds considered include cyromazine, melamine, hexamethylmelamine, anilazine, and s-triazines with an aziridine or 5-nitrofuryl moiety.

B. Selection of the Candidate Group

As outlined in the 1999 Common Mechanism Guidance document (US EPA, 1999a), the stepwise process of selecting a candidate group of chemicals starts with an initial grouping of chemicals selected based on having shared structural, toxicological and/or pesticidal properties. HED first examined the triazine pesticides, atrazine, simazine, and cyanazine, for inclusion in the initial common mechanism group. That examination showed that these pesticides shared both structural characteristics and toxicological endpoints. Structurally, all three pesticides contain the s-triazine moiety. Toxicologically, the pesticides are positive for mammary gland tumors in Sprague-Dawley (SD) rats, and atrazine and simazine have data suggesting that they interfere with the LH ovulatory surge.

HED then examined whether any other 1,3,5-triazines or other pesticides or metabolites containing s-triazine rings shared these characteristics. As shown in Table 1, four of the five 2-chloro-s-triazines, atrazine, simazine, propazine, and cyanazine, as well as metabolite DACT (Table 3), are positive for mammary gland tumors in female SD rats. Terbutylazine produced mammary gland tumors in Tif:RAlf female rats in addition to benign testicular tumors in males. Although 2-hydroxyatrazine does not produce mammary gland tumors, it has been found to produce some reproductive developmental effects consistent with atrazine.

Terbutryn produced mammary gland tumors (CR:CD rats), and, in addition, produced statistically significant increases in combined benign and malignant tumors in testis, thyroid, and liver (Table 1). Prometryn and ametryn were negative for rodent mammary gland tumors.

Among the methoxy s-triazines shown in Table 1, terbumeton was positive for mammary gland tumors, but it is not marketed in the United States. Prometon was negative for oncogenicity in rodents.

As shown in Table 2, only one sulfonyl urea herbicide (Express) produced statistically significant incidences of benign and combined benign/malignant mammary gland tumors in female SD rats. The effect was seen at a considerably higher dose (1250 ppm) compared to that observed for other s-triazines and no other tumors were observed. Because metabolism data for Express indicate that cleaved s-triazine products are seen in tissues and excreta of dosed female rats, the sulfonyl urea herbicide is included in the candidate group, based on its capacity to produce mammary gland tumors in female SD rats and evidence that a triazine moiety is released during metabolism of the

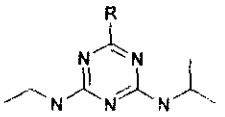
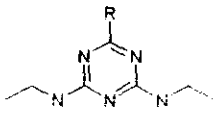
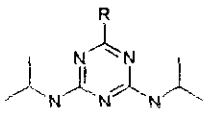
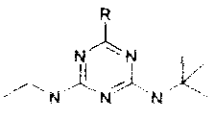
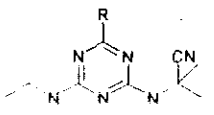
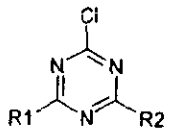
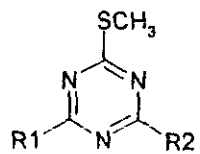
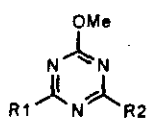
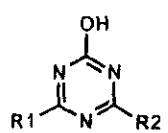
parent compound. No rodent mammary gland tumors were seen for the other four listed sulfonyl urea herbicides, metsulfuronmethyl (SD rats, up to 5000 ppm), trisulfuron (SD rats, up to 6000 ppm), chlorsulfuron (up to 2500 ppm), and harmony (CD rats, up to 2500 ppm).

Based upon OPP's review of the available toxicity information, a subset of eight pesticides containing the triazine moiety – atrazine, simazine, propazine, cyanazine, terbutylazine, terbumeton, terbutryn, tribenuron methyl (Express) – were found to cause the similar toxic effect of inducing mammary gland tumors only in female rats but not male rats or both sexes of mice. The other triazine-containing pesticides – ametryn, prometryn, prometon, metsulfuron methyl, trisulfuron, chlorsulfuron, DPX-M6316 (Harmony), cyromazine, melamine, hexamethylmelamine, anilazine, and s-triazines with an aziridine or 5-nitrofuryl moiety– do not cause that carcinogenic profile or their structures contain moieties that have a confounding effect as to their mechanism of toxicity, and there is no known mechanism of toxicity that would support grouping them by a common mechanism with atrazine, simazine, and cyanazine.

Further, only four of the subset of eight triazine-containing pesticides– atrazine, propazine, simazine, and Express – have uses that result in exposure to the general public; the other four– terbutylazine, terburmeton, cyanazine, and terbutryn – do not have tolerances and either are not registered or have registrations that do not involve exposure to the general public.

Thus, as shown in Table 3, the compounds being considered in making a determination about grouping pesticides via a common mechanism of toxicity are **atrazine, simazine, propazine, tribenuron methyl (Express)** and metabolites, **2-hydroxyatrazine, DACT, DEA, and DIA**, given their structures, ability to induce mammary gland tumors in female SD rats, and/or ability to affect LH-dependent events. This group hereafter will be referred to as the **candidate group**. The metabolites are specifically included because atrazine, simazine, and propazine break down to two or all of them and they are found as residues in drinking water and food. Toxicity data on the chloro-s-triazine metabolites also provide for supporting the common mechanism of toxicity for the parent compounds.

Table 1. SAR and Mammary Gland Tumor Induction by Various s-Triazines Compounds in Rats

					
	Atrazine + Positive for mammary gland tumors at 70 ppm	Simazine + Positive for mammary gland tumors at 100 ppm	Propazine + Positive for mammary gland tumors at 3 ppm	Terbutylazine + Positive for mammary gland tumors at 750 ppm[1]	Cyanazine + Positive for mammary gland tumors at 5 ppm
	Ametryn (–)	NE[3]	Prometryn (–) Negative for oncogenicity up to doses of 3000 ppm	Terbutryn + Positive for mammary gland tumors at 3000 ppm[2]	NE[3]
	NE[3]	NE[3]	Prometon (–) Negative for oncogenicity up to doses of 1000 ppm	Terbumeton +	NE[3]
	OH-Atrazine (–)	NE[3]	NE[3]	NE[3]	NE[3]

[1] Dose considered to be excessive, EPA Classification of D (not classifiable to human carcinogenicity). Study used Tif:RAlf rats . Benign interstitial cell tumors of the testes were also observed.

[2] Also statistically significant ($p \leq 0.03$) increase in combined benign and malignant tumors in testis, thyroid, and liver. [3] NE = not evaluated

Table 2. Mammary Gland Tumor Induction by Sulfonylurea Herbicides Containing the s-Triazine Moiety

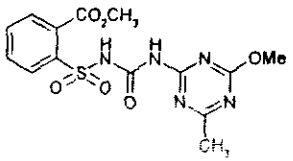
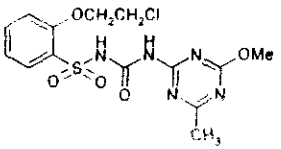
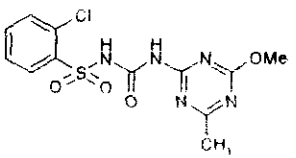
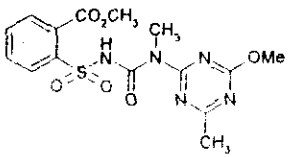
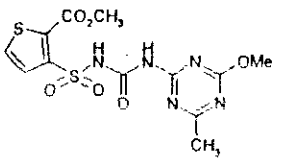
				
Metsulfuronmethyl (–)	Trisulfuron (–)	Chlorsulfuron (–)	DPX-L5300 (Express) + Positive for oncogenicity at 1250 ppm	DPX-M6316 (Harmony) (–)

Table 3. Structures of the Compounds in the Candidate Group

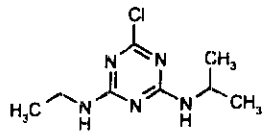
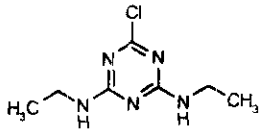
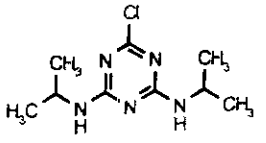
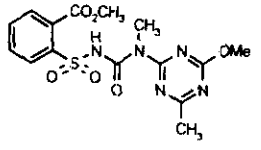
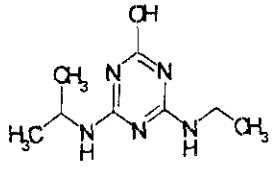
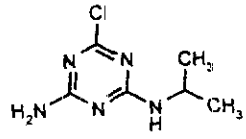
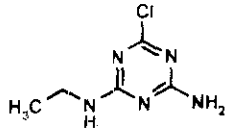
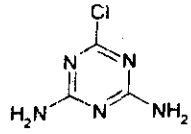
Compound	Structure	CAS No.	PC Code
Atrazine		1912-24-9	080803
Simazine		122-34-9	080807
Propazine		139-40-2	080808
Tribenuron-methyl (Express)		101200-48-0	128887
2-Hydroxyatrazine		2163-68-0	—

Table 3. Structures of the Compounds in the Candidate Group (Continued)

Compound	Structure	CAS No.	PC Code
Desethyl Atrazine (DEA)		6190-65-4	-
Desisopropyl Atrazine (DIA)		1007-28-9	-
Diaminochlorotriazine (DACT)		3397-62-4	-

III. Mechanism of Toxicity

This section describes the proposed mechanism for the common toxic endpoints by which the compounds containing the s-triazine moiety might be grouped. The triazine compound atrazine has been used as a prototype for defining the toxic effects of triazines and elucidating the mechanism of toxicity associated with these effects, given that it is the most extensively studied triazine. Multiple studies on the effects of atrazine have been published in open literature and conducted by registrants and EPA's National Health and Environmental Effects Research Laboratory (NHEERL). These studies demonstrate that the most relevant endpoint selected for intermediate-term and chronic risk assessments is a neuroendocrine effect exemplified in female rats by attenuation of the luteinizing hormone (LH) surge and accompanying disruption of the estrous cycle. In depth reviews and discussions of these studies may be found in the following documents:

1) Revised Preliminary Human Health Risk Assessment (http://www.epa.gov/pesticides/reregistration/atrazine/ revsd_pra.pdf) (US EPA, 2001a).

2) Atrazine: Toxicology Chapter of the Reregistration Eligibility Decision. REVISED, (http://www.epa.gov/pesticides/ reregistration/atrazine/tox_chapter.pdf) (US EPA, 2001b)

3) Hazard and Dose-Response Assessment and Characterization of Atrazine (Part A), Hazard Assessment and Review of Available Studies (Part B), and References, http://www.epa.gov/ scipoly/sap/2000/june27/ finalparta_atz.pdf and http://www.epa.gov/scipoly/sap/2000/june27/ finalpartb_atz.pdf (US EPA, 2000a; US EPA 2000b).

4) SAP Report No. 2000-05, Atrazine: Hazard and Dose-Response Assessment and Characterization, http://www.epa.gov/scipoly/sap/ 2000/ june27/ finalpartc_atz.pdf (US EPA, 2000c).

A few of the more recent and pertinent studies are reviewed in this document in order to establish this neuroendocrine effect as relevant to a toxic effect common among several of the compounds containing the s-triazine moiety and their metabolites, and to establish its mode of action. However, the reader is referred to the citations above for more extensive reviews.

The carcinogenic effects of atrazine have been clearly demonstrated. The earliest published study documenting these effects showed that there were dose-related increases in the incidence and/or early onset of mammary gland tumors (adenomas, adenocarcinomas, and carcinosarcomas combined) in female Sprague-Dawley (SD) rats in a seminal carcinogenicity test performed with atrazine (Mayhew *et al.*, 1986). No dose-related increases in tumor responses were observed in male SD rats. Results of subsequent bioassays, some of which included serial and/or one year

sacrifices, confirmed that the predominant response observed following testing of atrazine in female SD rats is an increase in the incidence and/or early onset of mammary gland adenomas/carcinomas. Less compelling evidence suggests that there is decreased latency for the formation of mammary gland fibroadenomas and pituitary adenomas (Thakur, 1991a and 1992a; Pettersen and Turnier, 1995) and an increased incidence of mammary gland fibroadenomas (Morseth, 1998). An increased tumor incidence is not found at any other site in female SD rats, or at any site in male SD rats, or in either sex of Fischer 344 rats and CD-1 mice (Mayhew *et al.*, 1986; Hazelette and Green, 1987; Thakur, 1992a,b). Mammary gland tumors were reported in one study in male Fischer 344 rats that involved lifetime treatment with atrazine (Pinter *et al.*, 1990), but the finding is difficult to evaluate in light of the experimental design and shortcomings of the study. Furthermore, this finding is in conflict with the results of a conventional 24-month carcinogenicity study with F344 male rats that showed no increases in mammary gland tumors (Thakur, 1992b). The closely related structural analogues to atrazine (i.e., simazine, propazine, and cyanazine) also produce mammary gland tumors in the female SD rat but no other tumors of any type in the female SD rat and no tumors of any kind in the male SD rat or in CD-1 mice of either sex.

As a result of the above-mentioned studies with atrazine, a central nervous system (CNS) mechanism of toxicity has been proposed for the increased incidence of mammary gland tumors. It is hypothesized that the carcinogenicity of atrazine is a consequence of the disruption of the normal secretory activity of the hypothalamic-pituitary-ovarian axis. Figure 2 illustrates the proposed mode of action of atrazine in female SD rats on the activity of the hypothalamic-pituitary-ovarian axis and the development of mammary gland and to some extent pituitary neoplasms. As depicted in Figure 2, atrazine exposure affects the hypothalamus, leading to a decreased secretion of hypothalamic norepinephrine (NE) (Cooper 1998). Decreased NE levels result in decreased release of gonadotropin releasing hormone (GnRH) from the hypothalamus (Cooper, 1998). GnRH is the hormone responsible for inducing the pituitary gland to release luteinizing hormone (LH). Thus, a decreased GnRH level leads to an attenuated LH release (Cooper *et al.*, 1995, 1996, 2000; Morseth, 1996a, b). LH normally provides a signal to the ovaries promoting ovulation, but under atrazine's exposure serum LH levels are insufficient to stimulate ovulation. Under the tonic secretion of LH and follicle-stimulating hormone (FSH), this feedback mechanism eventually causes the ovarian follicles to continue to secrete estradiol, which in turn leads to the hypertrophy of pituitary lactotrophs and consequently the increase in prolactin secretion. In concert with prolactin, estrogen acts on the mammary gland and increases the risk for mammary gland tumors.

Suppression of the LH surge in female SD rats is considered to be a necessary precursor for the development of atrazine-induced mammary gland tumors. This is because LH blood levels must reach a sufficient magnitude to induce ovulation and to maintain normal reproductive cycles. When atrazine reduces LH output to the critical point where there is not enough to trigger ovulation, a physiological state results which is characterized by prolonged or persistent estrus. This state leads to continued stimulation of mammary tissue by estrogen. Evidence for an attenuation of the LH

surge and an early onset of prolonged and/or persistent estrus is provided in several studies (Morseth 1996a,b; Thakur 1991a; Eldridge *et al.*, 1993). Removal of the estrogen stimulus by ovariectomy completely abolishes the formation of mammary gland tumors following chronic administration of atrazine (Morseth, 1998). Estrogen has been strongly implicated in mammary gland cell proliferation and the enhancement of neoplastic transformation in rodents and humans (for review see Russo and Russo, 1996; Nandi, 1995).

It should be noted, however, that the proposed carcinogenic mode of action for atrazine in rats is not likely to be relevant to humans. As summarized by the FIFRA Scientific Advisory Panel (SAP), "there are considerable differences between hypothalamic-pituitary-ovarian function in rats and humans, and the effects of aging on the function of the axis also is quite dissimilar. Therefore, it is unlikely that the mechanism by which atrazine induces mammary gland tumors in female SD rats could be operational in humans. Nevertheless, it is not unreasonable to expect that atrazine might cause adverse effects on hypothalamic-pituitary function in humans" (US EPA, 2000c). Although the cancer mode of action may not be operative in humans, the SAP went on further to state that "the same endocrine perturbations that induce tumors also appear to play a role in at least some reproductive developmental effects", which may be relevant to humans (See Figure 2).

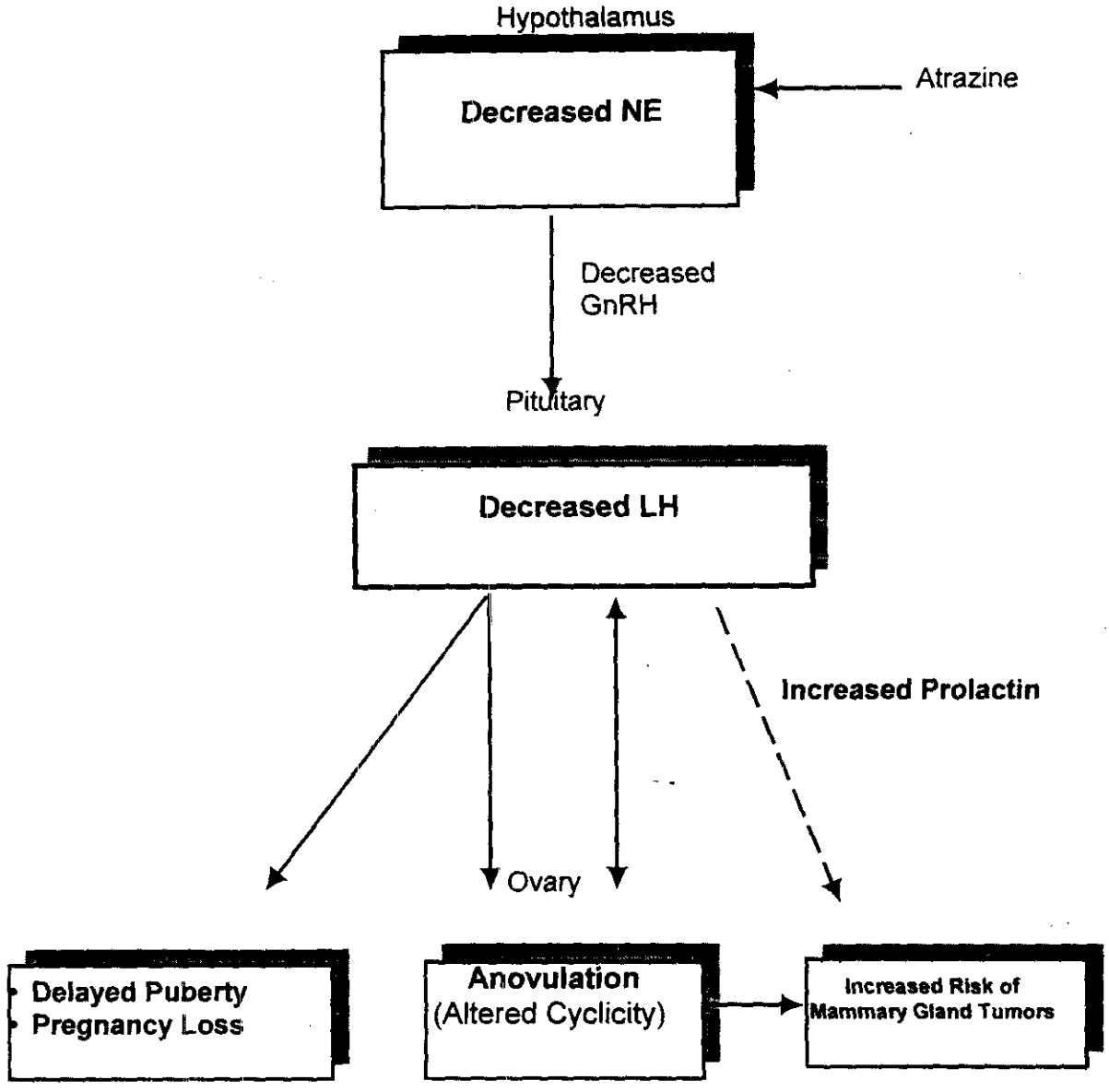
In addition to the disruption of the estrous cycle, the suppression of LH by atrazine has been found to be accompanied by adverse reproductive functions (discussed later in Section IV). Studies designed to evaluate the effect of atrazine on early pregnancy found that atrazine increased pre- and post-implantation loss and delayed parturition in various strains of female rats (Cummings *et al.*, 2000; Narotsky *et al.*, 2001). Further, it has been found that pubertal development is delayed in male and female Wistar rats administered atrazine (Laws *et al.*, 2000; Stoker *et al.* 2000 and in press).

Alternative modes of action for the neuroendocrine effects following exposure to compounds containing the s-triazine moiety have been suggested. Although several studies have found that the estrogenic effects associated with some of the compounds containing the s-triazine moiety *in vivo* are not estrogen receptor-mediated (Tennant *et al.*, 1994a,b; Conner *et al.*, 1996), these effects may be explained partly by their ability to induce aromatase, the enzyme responsible for converting androgens to estrogens. Recent studies demonstrated that atrazine, simazine and propazine, but not metabolites, DEA or DIA, induced aromatase activity in various cell lines (Sanderson *et al.*, 2001, 2000). Further, as raised by Trentacoste (2001) and by one member of the SAP (US EPA 2000c), it has been suggested that the anorexic effects of atrazine could account for most of atrazine's effect on LH since reduced food intake and weight loss is a potent stimulus for reduced LH secretion. However, in pair-fed studies in both males and females, decreased food consumption and body weight could not account for the adverse effects of atrazine on the estrous cycle and pubertal development (Laws *et al.*, 2000; Stoker *et al.*, 2000) Upon consideration of the SAP comments, OPP's own reviews and the data underlying these reviews, as well as additional information received by the Agency from registrants or presented in open literature, it has been

concluded that the neuroendocrine actions of atrazine are the primary and requisite mode of action for the induction of mammary gland tumors and certain reproductive developmental effects (see Figure 2).

Given the overall consistency and specificity of the evidence for atrazine's proposed mechanism of toxicity, studies on the effects associated with disruption of the hypothalamic-pituitary-gonadal axis have been conducted with the other compounds containing the s-triazine moiety (e.g., propazine, simazine, and certain chloro-s-triazine metabolites). Similar to atrazine, some of these compounds have been found to produce mammary gland tumors only in female SD rats and affect the hormonal control of reproductive functions. The attenuation of the pituitary LH surge and induction of reproductive developmental effects will be the basis upon which a common mechanism of toxicity will be determined for the candidate group, as established in the rest of this document.

Figure 2. Atrazine[1]: Neuroendocrine Mode of Action and Associated Effects Found in Rats.



[1]Atrazine also produced a decrease in pituitary prolactin, which also contributes to effects on reproductive development and affects lactation; NE = norepinephrine; LH=luteinizing hormone; GnRH = gonadotropin releasing hormone.

IV. Lines of Evidence

A. Structure Activity Considerations

In general, based on structure-activity relationships (SAR), the pesticides may be grouped according to their likelihood to generate a common type of toxic molecule or reactive intermediate or their ability to mimic a common biologically active molecule that interferes with the normal homeostasis of the cell (e.g., via receptor binding, enzyme induction, etc.).

As shown in Table 3, all compounds in the candidate group share an s-triazine ring in their structure. All compounds, except tribenuron methyl (Express) and 2-hydroxyatrazine, have a chlorine atom in the 2-position with alkyl amino groups at the 4- and 6- positions (atrazine, simazine, and propazine) or at either the 4- (DEA) or 6- (DIA) positions only. Diaminochlorotriazine (DACT) is the fully dealkylated triazine of the group. This compound is a common metabolite of atrazine, simazine, and propazine in the rat, and can, like its parental precursors, decrease the intensity of the LH surge in female rats.

Based on structure-activity considerations, it is reasonable to expect that three of the candidate herbicides, atrazine, propazine, and simazine may share common toxic effects, metabolic pathways, and mechanism(s) of action. Atrazine and propazine share a common metabolite, desethyl atrazine (DEA), while atrazine and simazine share desisopropyl atrazine (DIA) as a common metabolite. A further dealkylation of the desethyl- or desisopropyl atrazine yields diaminochlorotriazine (DACT), which is thus common to all three chlorotriazines. Atrazine can also lose its chlorine atom to form 2-hydroxyatrazine; however, as shown in Table 1, this compound does not induce mammary gland tumors in SD female rats.

B. Metabolism and Pharmacokinetics Considerations

Metabolism and pharmacokinetics considerations can play an important role in determining common mechanisms of toxicity in a candidate set of chemicals. Information on the disposition of a chemical helps to elucidate issues of target site dose delivery. The study of the biotransformation of the chemicals can determine if a putative common toxic metabolite or its precursor are produced.

As discussed below, the candidate group compounds have many metabolic similarities, as well as some differences.

1. Absorption

Absorption of the candidate group herbicides after oral dosing, as measured indirectly in laboratory studies, is significant and may impact the potential human dose from water and food exposures. Measurement of excretion of radioactivity in urine of rats (an approximate measure of absorption) for (14)-C-labeled atrazine demonstrated 67% of the dose was excreted through the urine (Timchalk *et al.*, 1990). The urinary excretion profiles for the some of the candidate group compounds are listed in Table 4.

The percentage of administered triazine dose excreted is similar for atrazine and propazine (Table 4). Excretion in urine for simazine was slightly smaller, 49.3% of the dose, and that of 2-hydroxyatrazine was slightly higher. Since Table 4 compares data from studies conducted under different protocols, it is difficult to assess the significance of differences in excretion times and profiles; however all of them are consistent with extensive absorption of the test material by the oral route.

Oral administration of the sulfonyl urea Express to Crl:CD:BR rats resulted in urinary excretion of over 60% of the dose.

Table 4. Urinary Excretion for (14)-C -s-Triazines by Orally Dosed Rats

Compound	Oral Dose	% Dose Excreted	Reference
Atrazine	~ 1.5 mg/kg	65.5 (72 hrs)	Bakke et al. (1972)
Atrazine	30 mg/kg	67 (72 hrs.)	Timchalk *et al.* (1990)
Atrazine	Unspecified	65 (72 hrs.)	Trochimowitz *et al.*, (1994)
Simazine	1.5 mg/kg	49.3 (96 hrs.)	Simoneaux and Sy (1971)
Propazine	1.0 mg/kg	69.5♂ /68.8♀ (7d)	Krautter (1995)
Propazine	41-56 mg/kg	66 (72 hrs.)	Bakke *et al.* (1967)
2-OH - Atrazine	~1.5 mg/kg	78 (72 hrs.)	Bakke et al. (1972)

2. Tissue Distribution

Tissue residue analysis in rats dosed with radiolabelled atrazine, simazine or propazine indicate extensive tissue distribution of radioactivity from these compounds to sites, including the brain. Paul et al. (1993) administered a single oral dose of ^{14}C -atrazine (1 mg/kg) to male SD rats. At 24 hours after dosing, percent of dose in heart, lungs, brain, liver,

and testes amounted to 0.22, 0.35, 0.49, 3.9, and 0.63 % of the dose, respectively. Radioautography of rats treated with a single oral dose of ^{14}C -atrazine (100 mg/kg) showed extensive distribution of label throughout the body, including the brain and adjacent tissues. Orr and Simoneaux (1986) administered a single oral dose of ^{14}C -simazine (0.5 mg/kg) to CD rats of both sexes. At 7 days after dosing, percent of dose in heart, lungs, brain, liver and uterus in females amounted to 0.04, 0.05, 0.10, 1.26, and 0.01 % of the dose, respectively. Corresponding values for males were 0.04, 0.06, 0.09, 1.08, and 0.01 (for testes), respectively. Bakke et al. (1967) administered a single oral dose of ^{14}C -propazine (~ 49 mg/kg) to male SD rats. At 2 days after dosing tissue residues in heart, lung, brain, liver and spleen amounted to 51, 51, 34, 52 and 47 ppm (as propazine equivalents), respectively.

3. Biotransformation

All of the candidate group compounds undergo extensive biotransformation in rats. As summarized below, numerous metabolites have been detected in both rat and human urine, many of which are the same. As illustrated for atrazine (Figure 3), the main biotransformation pathways for the chloro-s-triazines in rats are N-dealkylation by the hepatic cytochrome P450 system, and glutathione conjugation of either the parent or the N-dealkylated metabolite to the ultimately excreted mercapturic acid conjugate (Figure 3). Express, a sulfonylurea triazine, likewise undergoes extensive biotransformation.

a. Atrazine

The N-dealkylated urinary metabolites of atrazine in rats were quantitated by Bradway and Moseman (1982). As specified in Table 5, the major metabolite was diaminochlorotriazine (DACT). The minor metabolites, (desisopropyl s-triazine (DIA) and desethyl s-triazine (DEA), were detected in the higher dose groups.

Rat metabolism of atrazine was also studied by Timchalk *et al.* (1990). Fischer 344 rats were given a single oral dose of 30 mg (14)C-labeled atrazine per kg of body weight. The atrazine was quickly metabolized as the urine excreted within 24 hours of the dosing contained approximately 57% of the administered radioactivity. As shown in Table 5, the major urinary metabolite was DACT. The other reported urinary metabolites were DACT-mercapturate, DIA, DEA, and DEA-mercapturate. These metabolites were identified based upon similar HPLC retention times as synthesized standards (Timchalk *et al.*, 1990). Paul *et al.* (1993) found levels of DACT up to 25% of the dose in rats (Table 5).

As shown in Figure 4, atrazine, simazine, and propazine share N-dealkylation metabolic pathways and thus these three compounds have the metabolite diaminochlorotriazine (DACT) in common. As will be discussed later, DACT causes a decrease in the LH surge in SD female rats and produces effects on reproduction and development.

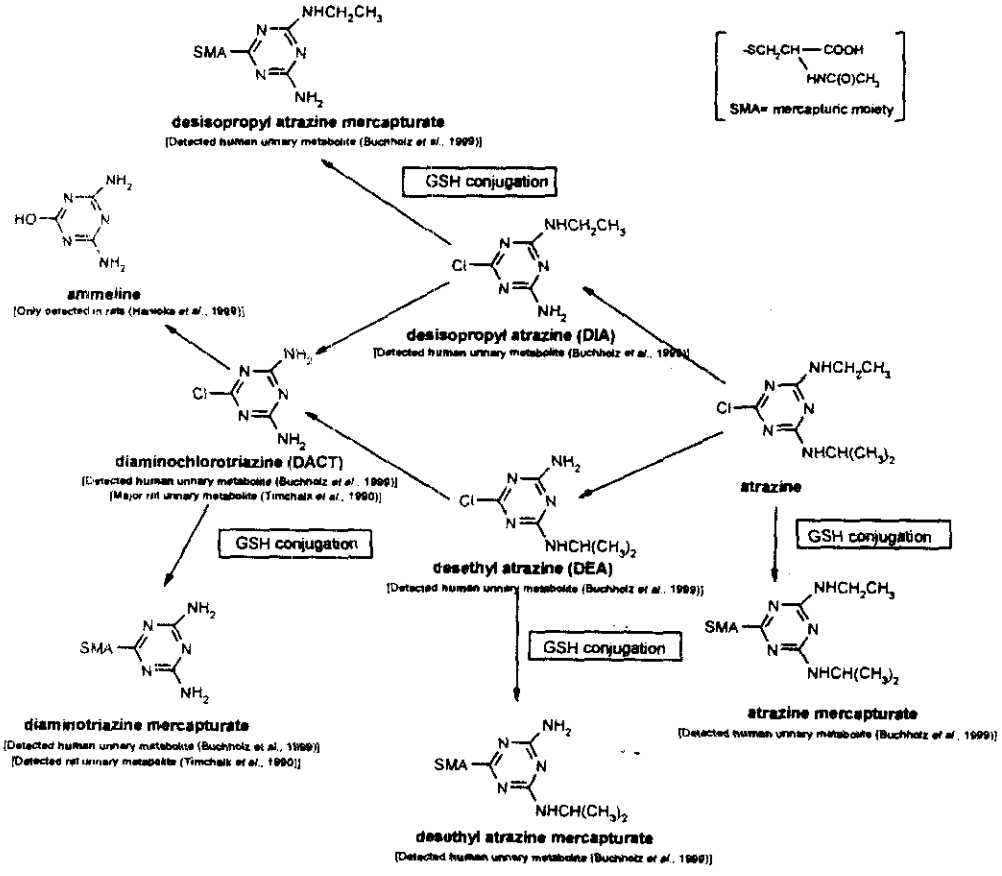


Figure 3. Biotransformation for Atrazine [Adapted from Buchholz *et al.* 1999, Hanioka *et al.* 1999, and Timchalk *et al.* 1990].

Table 5. Relative Percentage(s) of (14)-C Atrazine[2] Urinary Metabolites

Reference	Species	Route	Dose Level	Metabolite(s)[1]	% Administered Dose Excreted in Urine as Metabolite(s)	Notes
Bradway and Moseman, 1982	Rat Males F344	Oral, Single Dose	170 mg/kg	DACT DIA & DEA	Not Measured 3.7%	Results not reported separately for DIA & DEA. DACT not studied.
			17 mg/kg	DACT DIA & DEA	Not Measured 0.3%	
			1.7 mg/kg	DACT DIA & DEA	Not Measured Not Detected	
Bradway and Moseman, 1982	Rat Males F344	Oral, For Days 1, 2 & 3	17 mg/kg/day	DACT	3.2% (day 1), 31.9% (day 3)	No DACT recovered at doses of 0.17 & 0.017 mg/kg/day
			1.7 mg/kg/day	DACT	2.9 % (day 1) 4.3 % (day 3)	
Paul et al. 1993 (MRID 44713802)	Rat Males SD	Oral, Single dose	1 mg/kg	DACT DACT-mercapturate DIA DEA	25.8 % 1.1% 0.2 % 0.07 %	
			100 mg/kg	DACT DACT-mercapturate DIA DEA	14.2 % 2.5% 0.8 % 0.2 %	
Timchalk *et al.*, 1990	Rat Males SD	Oral Single dose	30 mg/kg	DACT DACT-mercapturate DIA DEA-mercapturate DEA	39% (67%)[3] 5% (9%)[3] <0.6% (<1%)[3] 8% (13%)[3] 3% (5%)[3]	Data from urine extracts from 0 to 24 hours after dose

[1] See Figures 3 and 4 for structural identity of metabolites
[2] Bradway and Moseman used non-radiolabelled atrazine
[3] Percentage of total urinary radioactivity

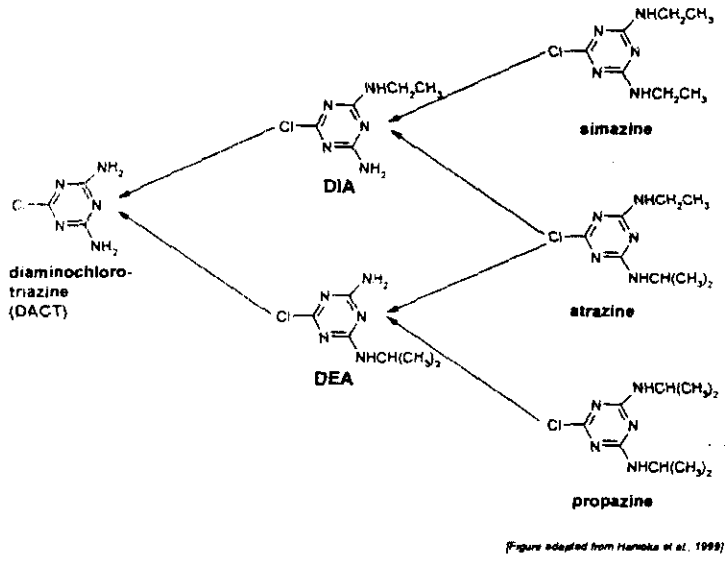


Figure 4. Dealkylation of Simazine, Atrazine, and Propazine

b. Propazine

As summarized in Table 6, the dealkylated urinary metabolites of propazine in rats were quantitated by Bradway and Moseman (1982). The major metabolite was DACT; and a second metabolite, DEA, was detected in the higher dose groups (Bradway and Moseman, 1982).

c. Simazine

The dealkylated urinary metabolites of simazine in rats were quantitated by Bradway and Moseman (1982). As specified in Table 6, the major metabolite was DACT. A second metabolite, DIA, was detected at a lower level (Bradway and Moseman, 1982).

Table 6. Relative Percentage(s) of Propazine and Simazine Urinary Metabolites

Compound	Reference	Species	Route	Dose Level	Metabolite(s)[1]	% Administered Dose Excreted in Urine as Metabolite(s)
Propazine	Krautter, 1995 (MRID 43689801)	Rat Male & Female SD	Oral Single Dose	1 mg/kg	DACT 2-OH-DEA DEA	28.8 % ♂, 26.9 % ♀ 2.6% ♂, ND ND ♂, 0.7% ♀
				100 mg/kg	DACT 2-OH-DEA DEA	28.2 % ♂, 19.9 % ♀ ND ♂, ND ♀ 0.9% ♂, ND ♀
Propazine	Bradway and Moseman, 1982	Rat Males F344	Oral Single dose	170 mg/kg	DACT DEA	Not Measured 0.5%
				17 mg/kg	DACT DEA	Not Measured 0.08%
Propazine	Bradway and Moseman, 1982	Rat Males F344	Oral, For Days 1, 2 & 3	17 mg/kg/day	DACT	3.3% (day 1) 9.6% (day 3)
				1.7 mg/kg/day	DACT	0.34% (day 1) 17% (day 3)
Simazine	Bradway and Moseman, 1982	Rat Males F344	Oral Single dose	170 mg/kg	DACT DIA	Not Measured 2.8%
				17 mg/kg	DACT DIA	Not Measured 0.5%
				1.7 mg/kg	DACT DIA	Not Measured 0.4%
Simazine	Bradway and Moseman, 1982	Rat Males F344	Oral, For Days 1, 2 & 3	17 mg/kg/day	DACT	3.9% (day 1) 18.2% (day 3)
				1.7 mg/kg/day	DACT	1.4% (day 1) 1.6% (day3)

[1] See Figures 3 and 4 for structural identity of metabolites

d. Tribenuron methyl (Express)

As shown in Figure 5, Express undergoes extensive biotransformation in rats. In Crl:CD:BR rats dosed orally with ^{14}C-triazine-ring labeled Express recoveries of O-demethyl triazine amine, N-demethyl triazine amine and triazine amine in female urine amounted to 12.2, 3.4 and 3.4 percent of the dose. These values suggest extensive metabolic release of the triazine moiety in the dosed animals.

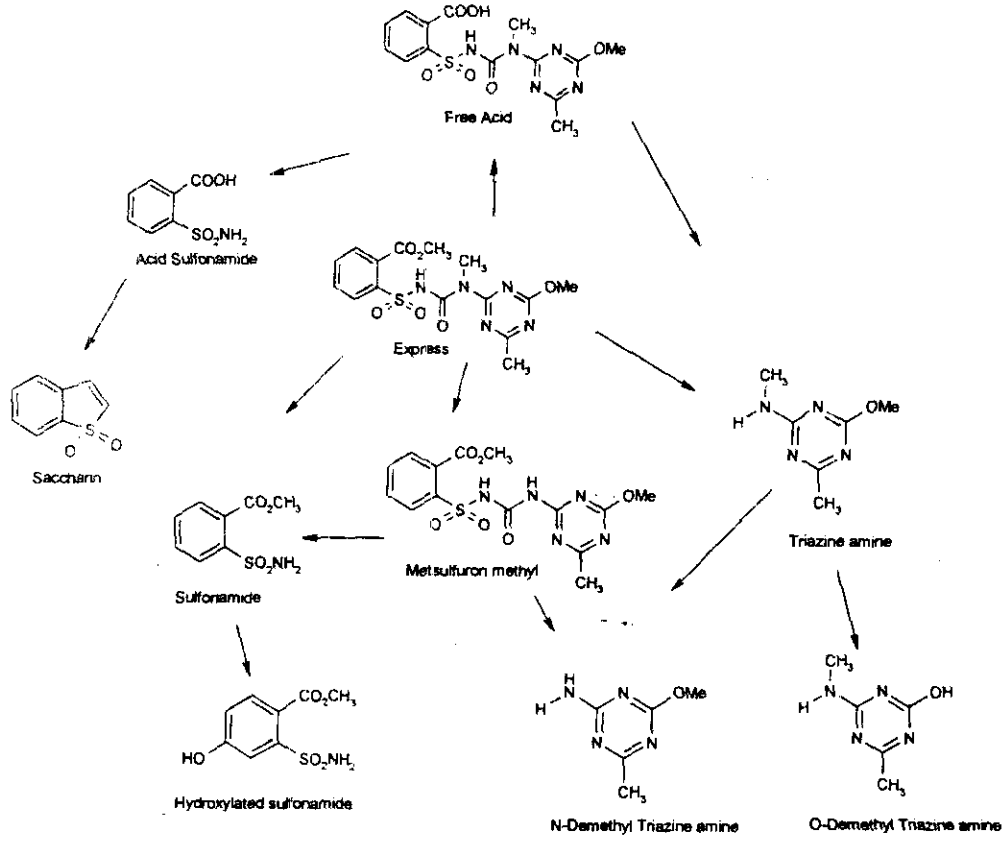


Figure 5. Biotransformation of Express in Rats (Adapted from MRID 40245516)

4. Summary

The previous sections describe the absorption, biotransformation and excretion profiles for the candidate compounds and their metabolites. All of the candidate group compounds are significantly absorbed by rats when administered orally. The main biotransformation pathways identified through excretion profiles involve hepatic cytochrome P450-mediated N-dealkylation and glutathione conjugation of either the dechlorinated or the N-dealkylated metabolite. Biotransformation of atrazine, simazine, and propazine, but not Express, results in the common metabolites DACT and/or DIA and DEA.

C. Toxicological Considerations

The identification of a candidate group of chemicals for a cumulative risk assessment involves an evaluation of effects that may be common to the group of chemicals under review. Following is a discussion of the relevant types of effects reported to be induced by treatment of laboratory animals with compounds containing a triazine moiety and an evaluation of the extent to which the effects are common to this group of chemicals. Hematological and cardiac effects following exposure to compounds containing a triazine moiety are not consistent and not associated with disruption of the hypothalamic-pituitary-gonadal axis, and therefore are not included in this discussion. Furthermore, developmental effects (e.g., incomplete or delayed ossification) are not discussed because there are no data that would suggest that the delays in ossification in fetal animals are due to disruption of the hypothalamic-pituitary-gonadal axis or any other common mechanism by the candidate group compounds. Although the disruption of the hypothalamic-pituitary-axis is plausible in humans, data from human studies are insufficient to rely on for this common mechanism assessment. Thus, this analysis only utilizes data from laboratory animals as a basis of grouping these compounds by a common mechanism of toxicity. The effects likely related to neuroendocrine disruption observed in *in vivo* studies with the candidate group compounds are summarized in Table 7 and discussed below.

Table 7. Neuroendocrine Effects Following Exposure to the Candidate Group

Toxic Effects	Atrazine	Simazine	Propazine	Express	HA[1]	DACT	DIA	DEA
Carcinogenicity								
Increased incidence of mammary gland tumors	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	no data	no data
Reproductive Developmental and Neuroendocrine								
Disruption of the estrous cycle	**Yes**	no data	no data	**Yes**	no data	**Yes**	no data	no data
Attenuation of LH surge	**Yes**	**Yes**	**Yes**	no data	no data	**Yes**	no data	no data
Attenuation of prolactin surge	**Yes**	no data	no data	no data	no data	no data	no data	no data
Delayed vaginal opening	**Yes**	no data	**Yes**	no data	**No**	**Yes**	no data	no data
Altered pregnancy outcome	**Yes**	no data	no data	no data	**Yes**	**Yes**	**Yes**	**Yes**
Delayed preputial separation	**Yes**	no data	no data	no data	**Yes**	**Yes**	**Yes**	**Yes**
Decreased testosterone	**Yes**	no data	no data	no data	no data	**no effect**	**Yes**	**no effect**
Decreased prostate weight	**Yes**	no data	no data	no data	no data	**Yes**	**Yes**	**Yes**
Prostatitis in offspring	**Yes**	no data	no data	no data	no data	no data	no data	no data
Increase or decrease in testes weight	**Yes**	**Yes**	**Yes**	no data	no data	**no effect**	**Yes**	**Yes**

[1] HA = 2-hydroxyatrazine

1. Carcinogenic Effects

Studies submitted to OPP report that mammary gland tumors in female rats are a characteristic common effect in studies conducted with candidate group compounds. The studies provide evidence that administration of these compounds to female SD rats leads to increased incidence and/or early onset of benign and mammary gland carcinomas and adenomas, mammary gland fibroadenomas, and pituitary adenomas (Table 8). As discussed earlier, mammary gland tumors are not likely to be relevant to humans. However, because mammary gland tumors are associated with attenuation of the LH surge they are an indicator of the common mechanism of toxicity.

As shown in Table 8, the carcinogenicity of atrazine in the female Sprague-Dawley (SD) rat has been confirmed in several two-year bioassays. These studies show that atrazine exposure results in an increased incidence and an early onset of mammary gland tumors in female SD rats (Mayhew *et al.*, 1986; Thakur, 1991a, 1992a; Pettersen and Turnier, 1995). However, no tumor response is seen in SD male rats, Fischer 344 rats, or CD-1 mice of either sex (Hazelette and Green, 1987; Thakur, 1991b; Thakur, 1992b). The lowest dose of atrazine associated with an increased incidence in mammary gland carcinomas is 3.5 mg/kg/day (Mayhew *et al.*, 1986).

Similar findings are also seen with simazine and propazine. In a combined chronic/carcinogenicity study, simazine at dose levels of 100 ppm (5.3 mg/kg/day) and 1000 ppm (45.8 mg/kg/day) resulted in a statistically significant dose-related trend in mammary gland carcinomas (McCormick *et al*, 1988). A higher incidence of mammary gland carcinomas was also seen in the recovery substudy (52 weeks of treatment with 1000 ppm followed by 52 weeks of recovery) for the control and high dose group (1/10 vs 4/10, respectively). Propazine at low and high doses was found to increase the incidence of mammary gland tumors in female SD rats following the administration of propazine in the diet at 0, 3, 100, or 1000 ppm for 2 years (Jessup, 1980).

In a chronic/carcinogenicity study Express was fed to SD rats in the diet at 0, 25, 250, and 1250 ppm for 2 years (Tobia, 1987). A statistically significant increase in mammary gland adenocarcinomas was observed in the highest dose group (1250 ppm) after two years of Express exposure. The LOAEL for this study was 250 ppm, based on body weight gain, and the NOAEL was 25 ppm.

There have been no studies submitted to the Agency on the carcinogenicity of the triazine metabolites DEA and DIA. Recent data submitted to OPP in a draft report indicate that DACT at 200 ppm increased the incidence of mammary gland tumors in female SD rats

(Minnema, 2002). There were no effects of DACT on mammary gland tumor incidence at feeding levels equal to or less than 25, 50, or 70 ppm. There was no increase above control levels in the incidence of mammary gland tumors or tumors of any type in a two-year chronic/carcinogenicity study on 2-hydroxyatrazine (Chow and Hart, 1995).

Table 8. Summary of Female Mammary Gland Tumor Incidence/Onset in Chronic Rat Bioassays Using Candidate Group Compounds

Study	Species/ Strain	Duration	Mammary Gland Tumor Incidence	Mammary Gland Tumor Onset
Mayhew *et al.*, 1986	Rat/SD	2 year (atrazine)	Statistically-significant increase in female carcinomas at 3.5 mg/kg/day when adjusted for survival	Not determined in this study
Thakur, 1991a	Rat/SD	2- year with serial sacrifices (atrazine)	A significant positive trend for fibroadenomas is seen.	The percentage of carcinomas occurring in the first year of the study was 0 in controls, 33% at 4.23 mg/kg/day, and 50% at 26.23 mg/kg/day.
Thakur, 1992a	Rat/SD	2- year (atrazine)	No statistically-significant increases in female fibroadenomas or carcinomas seen at either 3.79 or 24.01 mg/kg/day	The percentage of carcinomas and adenomas occurring in the first year of the study in controls was 0% while at 3.79 mg/kg and 23.01 mg/kg/day 27.3 and 33.3% of the carcinomas appeared in the first year of the study.
Pettersen and Turnier, 1995	Rat/SD	1-year (atrazine)	six carcinomas/adenomas and four fibroadenomas are seen at the 23.9 mg/kg/day group compared to one carcinoma and two fibroadenomas in the control group.	The increased incidence of tumors at one year indicates an earlier onset.
McCormick *et al.*, 1988	Rat/SD	2-year (simazine)	Statistically significant increase in female carcinomas and fibroadenomas at 100 ppm and 1000ppm	A higher incidence of mammary gland carcinomas was observed in the recovery group (52 weeks of treatment, followed by 52 weeks of recovery)
Jessup, 1980	Rat/SD	2-year (propazine)	Mammary gland tumors (adenocarcinomas and adenomas) were increased in low- and high-dose female groups (3 and 1000ppm).	Not altered in propazine exposed animals
Tobia, 1987	Rat/SD	2-year (Express)	Statistically significant increase in mammary gland adenocarcinomas was observed in the highest dose group (1250 ppm)	Not altered in Express exposed animals
Minnema, 2002	Rat/SD	1-year (DACT)	Statistically significant increase in the incidence of mammary gland tumors at 200 ppm.	Not determined in this study

2. Reproductive Developmental and Neuroendocrine Effects

Effects on Females

As shown in Table 9a, the candidate group of compounds have all been found to produce reproductive developmental effects in female rats. Some of these effects include an attenuation of the LH surge and disruption of the estrous cycle. Other effects observed include attenuation of prolactin release, altered pregnancy outcome, and delayed puberty in male and/or female rats. Although Express has been found to act as an estrogen agonist (Cook, 1989), the reproductive developmental effects of atrazine, simazine, and DACT (Tennant *et al.*, 1994a,b; Conner *et al.*, 1996) does not appear to be a result of estrogenic activity. The estrogenic activity of propazine has not been determined.

Evidence for an attenuation of the LH surge following exposure to compounds containing the s-triazine moiety is provided in several studies (Cooper, unpublished data; Minnema, 2001ab; Cooper *et. al.*, 1995,1996, 2000; Cummings *et al.*, 2000; Morseth 1996ab). In a recent study submitted to US EPA, Minnema (2001a) compared the effects of simazine, DACT, and atrazine on LH. Simazine, DACT, and atrazine were administered to 20 Sprague-Dawley Crl:CD BR female rats/dose/group by oral gavage at dose levels of 0, 2.5, 5, 40, 200 mg/kg/day once daily for at least 4 weeks. Results showed that all three compounds had similar effects on diminishing the peak LH when the peak for each animal was determined and time axis for the individual animal data was rescaled to zero time. All three compounds at the two highest doses, 40 and 200 mg/kg/day, significantly decreased adjusted peak LH surge. Moreover, unpublished data by US EPA laboratory (Cooper, unpublished data) show that propazine (300.0 mg/kg/day) decreases the LH surge by over 50% of control. However, a pilot study submitted to OPP comparing propazine with atrazine and DACT found that propazine (320.1 mg/kg/day) decreased the peak LH surge to only 78.7% of control, whereas the mean LH peak surges were decreased to 34.5 and 47.6% of control following atrazine and DACT, respectively (Minnema, 2001b).

When the LH surge is analyzed using the maximum increase in plasma LH over baseline level (LHMax), hour at which peak surge of LH occurred (TimeMax), and area under the curve for LH verses time profile (AUC), the effects of the simazine, DACT, and atrazine on LH surge differ. Evaluation of LHMax, TimeMax, and AUC in a 28-day oral gavage study found that simazine at 40 and 200 mg/kg/day and DACT at 200 mg/kg/day significantly decreased LHMax and AUC (Minnema 2001a). Atrazine had no effects on any of the parameters at any dose level. Further, in a one-year chronic study with atrazine, DACT, and

simazine, attenuation of LH surge, as measured by LHMax and AUC, was only observed at the highest dose level (1854 µmoles/kg feed) of DACT (Minnema, 2002). Hence, the use of these parameters as measures of LH surge yield inconsistent data with previous reports and may not represent an accurate measure of the effects of these compounds on LH surge. It should be noted that a recently submitted preliminary report on the effects of atrazine in monkeys on LHMax, rate of rise of LH, AUC, and TimeMax showed inconclusive evidence of atrazine's adverse effects on LH after either 5 or 26 days of treatment due to several confounding factors (e.g., a limited sample size and a higher degree of individual variability in LH measurements than expected) (Parshley, 2001).

The effects of candidate group compounds on reproductive and neuroendocrine functions have been further characterized by cyclicity, pregnancy outcome, and pubertal developmental studies. Atrazine and DACT have been shown to prolong the duration of the estrous cycle at relatively low doses (Morseth, 1996ab; Pettersen *et al.*, 1991). Additionally, Express has been shown to slightly prolong the estrous cycle, in addition to decrease the estrogen-binding affinity of receptors in the uterus and mammary glands, at a very high dose (5000 ppm) (Cook, 1989). It can be expected that, if the LH surge and/or the estrous cycle are affected by exposure to candidate group compounds, then puberty and/or pregnancy may also be affected. In fact, studies conducted by US EPA labs have shown that atrazine at 50, 100, and 200 mg/kg delayed vaginal opening 3.4, 4.5, or greater than 6.8 days and produced irregular cycles in female Wistar rats (Laws *et al.*, 2000). More recently, it was reported that propazine and DACT, but not 2-hydroxyatrazine, delayed vaginal opening by up to 4 and 7 days, respectively (Laws *et al.*, 2002). Although the effects of atrazine and the metabolites (i.e., 2-hydroxyatrazine, DACT, DEA, and DIA) on pregnancy outcome varies considerably based on rat strain, they have been found to induce pre- and post-implantation loss, full litter resorption and delayed parturition (Narotsky *et al.*, 2002, 2001; Cummings *et al.*, 2000). See Table 9a for a summary of these effects.

As previously mentioned, although Express induces mammary gland tumors and appears to affect the ovarian cycle in female rats, limited data suggest that it is an estrogen receptor agonist. In a subchronic study (Cook, 1989), female Crl:CD®BR rats (20/dose level) were fed Express at 0 or 5000 ppm (approximately 390 mg/kg bw/day) for 84 days. At termination, the rats fed 5000 ppm had statistically significantly reduced body weights and body weight gains with respect to controls. Mean relative organ weights for treated rats terminated in estrus were significantly higher than in controls for liver (35% higher), uterus (31%), and ovaries (29%); mean relative organ weights for rats terminated in diestrus were also statistical significantly higher than in

controls for liver and uterus. In addition, the number of rats with a prolonged estrus, number of rats with 2 or more prolonged estrous cycles, and the number of cycles with a prolonged estrus were statistical significantly elevated at 5000 ppm *vs* controls. Furthermore, using radiolabelled thymidine incorporation, it was shown that cell proliferation in the uterus was increased in the rats treated with 5000 ppm. The ability of Express and its metabolites to compete *in vitro* for binding to the estrogen receptors in the uterus was further demonstrated when Express (ester and acid, see Figure 6 for structures) and its metabolites (N-demethyl triazine amine, N-demethyl-6-hydroxymethyl-triazine amine, α-hydroxytriazine amine, sulfonamide urea and metsulfuron methyl; all at 1.0 mM) competed with Diethylestilbestrol (0.125 mM) for binding to the estrogen receptor. None of these competed significantly with R5020 (0.125mM) for binding to the progesterone receptor.

Effects on Males

The candidate group compounds appear to not have a consistent effect on male gonadal weight. Atrazine, simazine, propazine and/or their metabolites, DIA, DACT, and DEA have been shown in different studies to increase, decrease or have no effect at all on testes weight (Mainiero *et al.*, 1987; Tai *et al.*, 1985; Gerspach, 1991; Jessup, 1979; Thompson *et al.*, 1992). However, atrazine and the metabolites have been found to delay the onset of puberty in male rats (Stoker *et al.*, in press; Stoker *et al.*, 2000). Stoker *et al.* (in press) also demonstrated recently that DACT, DIA, and/or DEA reduced ventral and lateral prostate, seminal vesicle, and epididymal weights when administered PND 23 through 53. Furthermore, when atrazine was administered to peripubertal male SD rats (22 to 47 days of age) at doses of 1 to 200 mg/kg/d, serum and intratesticular testosterone levels were reduced in the 100 and 200 mg/kg/d groups, as were seminal vesicle and ventral prostate weights (Trentacoste *et al.*, 2001). In the same study, serum LH was also reduced, suggesting an effect on the hypothalamus, the pituitary gland or both. Deprivation of prolactin during the early postnatal stage in the male offspring of dams receiving >25 mg/kg/d atrazine resulted in an increased incidence and severity of prostate inflammation (Stoker *et al.*, 1999). See Table 9b.

Table 9a. Lowest NOAELs/ LOAELs (mg/kg/day) for Reproductive Developmental Effects Following Exposure to Candidate Group Compounds in Female Rats

Response	Rat Strain	Exposure Period	NOAEL/LOAEL	Reference
FEMALE				
Attenuation of LH surge	LE	Single dose	300 (propazine)	Cooper, unpublished data
	SD	7 single daily doses	320.1 (propazine) 300 (atrazine) 202.4 (DACT)	Minnema, 2001b
	SD	28 daily doses	not determined (simazine)* not determined (DACT)* not determined (atrazine)*	Minnema, 2001a
	LE LE LE SD	1 day dose 3 daily dose 21 daily dose 21 daily dose	200/300(atrazine) <50/50 (atrazine) <75/75 (atrazine) 75/150 (atrazine)	Cooper *et al.*, 2000
	HLZ LE	GD 1-8	50/100 (atrazine)	Cummings *et al.*, 2000
	SD	28 days	5/40 (atrazine)	Morseth, 1996a
	SD	6 months	1.8/3.65 (atrazine)	Morseth, 1996b
Altered pregnancy maintenance	F344 SD LE	GD 6-10	25/50 (atrazine) 100/200 (atrazine) 100/200 (atrazine)	Narotsky *et al.*, 2001
	F344 HLZ	GD 1-8 GD 6-10	50/100 (atrazine) 50/100 (atrazine)	Cummings *et al.*, 2000
	F344	GD 6-10	25/50 (atrazine) 34/68 (DACT) 87/131 (DEA) 40/80 (DIA) <91/91 (hydroxyatrazine)	Narotsky *et al.*, 2002
Delayed parturition	F344	GD 6-10	50/100 (atrazine) 17/34 (DACT) <44/44 (DEA) 40/80 (DIA) 457/>457 (hydroxyatrazine)	Narotsky *et al.*, 2002
	F344 SD LE	GD 6-10	50/100 (atrazine) 50/100 (atrazine) 200/>200 (atrazine)	Narotsky *et al.*, 2001
Delayed vaginal opening	Wistar	PND 22-41	16.5/33.7 (DACT) 53/107 (propazine)	Laws *et al.*, 2002
	Wistar	PND 22-41	25/50 (atrazine)	Laws *et al.*, 2000

* These data are still under review by OPP.

Table 9a continued

Response	Rat Strain	Exposure Period	NOAEL/LOAEL	Reference
Disruption of estrous cycle	Wistar	PND 22-41	25/50 (atrazine)	Laws *et al.*, 2000
	SD	26 weeks	50ppm/400ppm (atrazine)	Eldridge *et al.*, 1999
	SD	28 days	5/40 (atrazine)	Morseth, 1996a
	SD	6 months	1.8/3.65 (atrazine)	Morseth, 1996b
	SD	13 weeks	10/100 (DACT)	Pettersen *et al.*, 1991
	SD	13 weeks	0/5000 ppm (Express)	Cook, 1989
Attenuation of prolactin release	LE LE LE SD	Adult females single dose 3 daily doses 21 daily doses 21 daily doses	atrazine: 200/300 serum <50/50 pituitary <75/75 pituitary <75/75 pituitary	Cooper *et al.*, 2000
Dams prolactin decreased	Wistar	PND 1-4	13/25 (atrazine)	Stoker *et al.*, 1999

Table 9b. Lowest NOAELs/ LOAELs (mg/kg/day) for Reproductive Developmental Effects Following Exposure to Candidate Group Compounds in Male Rats

Response	Rat Strain	Exposure Period	NOAEL/LOAEL (mg/kg/day)	Reference
Males				
Decreased LH	SD	PND 22-47	100/200 (atrazine)	Trentacoste *et al.*, 2001
Decreased testosterone & prostate weight	SD	PND 22-47	50/100 (atrazine)	Trentacoste *et al.*, 2001
Delayed preputial separation	Wistar	PND 23-53	12.5/25 (DEA) 12.5/25 (DIA) 6.25/12.5 (DACT)	Stoker *et al.*, 2002 (in press)
	Wistar	PND 23-53	<12.5/12.5 (atrazine)	Stoker *et al.*, 2000
	Wistar	PND 23-53	<11.4/11.4 (hydroxyatrazine)	Stoker, unpublished data
Increased incidence of prostatitis in offspring	Wistar	PND 1-4	13/25 (atrazine)	Stoker *et al.*, 1999
Increased incidence and severity of prostatitis in offspring	Wistar	PND 1-4	25/50 (atrazine)	Stoker *et al.*, 1999

V. Weight-of-Evidence Evaluation for Grouping the Candidate Group by a Common Mechanism of Toxicity

Table 10 lists the key LH-dependent effects that are considered to be relevant in defining those candidate group compounds that can be considered to have a common mechanism of toxicity due to disruption of the hypothalamic-pituitary-gonadal axis (see Table 7 for additional neuroendocrine toxic effects following exposure to the candidate group compounds). The relevant lines of evidence for grouping are discussed in the following pages. The common toxic effects of the candidate group compounds whose toxic effects have not been fully established is inferred based on metabolism data.

Table 10. Evidence Used in Grouping/Excluding Candidate Group Pesticides by a Common Mechanism of Toxicity[1]

Chemical	Mammary gland tumors	Suppress LH	Alter Cyclicity	Delay puberty	Alter pregnancy maintenance	Estrogen agonist
Atrazine	Yes	Yes male and female	Yes	Yes male and female	Yes	No
Simazine	Yes	Yes	No data	No data	No data	No
Propazine	Yes	Yes	No data	Yes	No data	No data
Express	Yes	No data	Yes	No data	No data	Yes
2-Hydroxyatrazine	No	No data	No data	No (females) Yes (males)	Yes	No data
DACT	Yes	Yes	Yes	Yes male and female	Yes	No
DEA	No data	No data	No data	Yes male	Yes	No data
DIA	No data	No data	No data	Yes male	Yes	No data

[1]**Effects are observed in females unless otherwise noted.**

A. Mammary Gland Tumors

A mechanism for the development of mammary gland tumors in female SD rats treated with **atrazine** has been detailed in this document and in previously cited documents. In summary, mammary gland tumors in the female rat result from a disruption of hypothalamic neurotransmitter and neuropeptide (primarily noradrenergic) regulation of GnRH, and subsequently, LH secretion. The resultant endocrine milieu of enhanced or unopposed estrogen and prolactin secretion provides an environment that is conducive to the development of mammary gland tumors.

Among the compounds listed in Table 10, **atrazine, simazine, propazine, Express**, and the metabolite **DACT** have been found to produce mammary gland tumors in rats.

- Atrazine, simazine, and propazine are not only structurally very similar, but present the same pattern of species/strain of mammary gland tumors, i.e. all three produce mammary gland tumors in the female SD rat but no other tumors of any type in the female SD rat, male SD rat, or in CD-1 mice of either sex.

- **DACT,** a metabolite of atrazine, simazine, and propazine, at 200 ppm increased the incidence of mammary gland tumors in female SD rats following one year of exposure (Minnema, 2002).

- **Express** produced mammary gland tumors in female Tif:RAlf rats. However, it is not clear that the same LH-related mechanism is operative in this compound as it is for atrazine because results from *in vivo* experiments (e.g., increased uterine cell proliferation & increased relative uterine weights) and *in vitro* experiments (e.g., estrogen receptor binding) with Express and its metabolites suggest that Express acts as an estrogen agonist. Thus, given its estrogenic activity, Express **can not** be grouped based on a common mechanism of toxicity and will consequently be excluded from the common mechanism group.

B. Attenuation of LH Surge

Studies have shown that **atrazine, simazine, propazine** and the metabolite **DACT** suppress the LH surge in rats (e.g., SD, Long-Evans). Atrazine suppresses LH in both male and female animals. The proximal effects of atrazine that lead to decreased LH levels outcomes have been identified as decreased hypothalamic norepinephrine levels and diminished ability to release gonadotropin releasing hormone from the hypothalamus (Cooper *et al.*, 1998). Atrazine has also been found by these same authors to increase hypothalamic dopamine and subsequently decrease prolactin secretion. As previously described, these neuroendocrine alterations can produce a cascade of effects which may alter the excitatory and inhibitory pathways and feedback loops essential for hormonal control in the hypothalamic-pituitary-gonadal (HPG) axis. Alteration of these pathways following exposure to compounds containing the triazine moiety have been found to exert effects on hormonal control of the estrus cycle in females, pubertal development in both males and females, pregnancy outcome, and prolactin secretion in laboratory rats. Although there are no direct data indicating that **DEA** and **DIA** attenuate the LH surge, it appears reasonable to expect that they will do so since **DACT**, an LH surge attenuator, is a metabolite common to both DEA and DIA in rats. This contention is supported by data that show that both **DEA** and **DIA** alter pregnancy maintenance (see below), an effect that is attributed to interference with the hypothalamic-pituitary-gonadal axis.

C. **Alteration of the Estrous Cycle**

Atrazine, **DACT, and Express** have all been shown to disrupt the estrous cycle by prolonging the number of days in estrus. Atrazine can increase the percentages of days in estrus by as much as 70%. Dietary exposure to DACT for 13 weeks induced irregularities of the cycle, which included early, intermittent, or persistent proestrus, estrus, and diestrus. Although **Express** has been shown to slightly prolong the estrous cycle, results of *in vivo* experiments showed that this compound possesses estrogenic activity, and therefore may not be acting by the same mechanism as atrazine, simazine, and propazine, as mentioned earlier in this section

D. **Delayed Pubertal Development**

Atrazine, **propazine**, and metabolites **2-hydroxyatrazine**, **DACT, DEA,** and **DIA**) have been found to delay pubertal development in rats. Atrazine and DACT delay puberty in both male and female rats, while 2-hydroxyatrazine has been found to delay puberty in males but not females. In females, the administration of propazine from postnatal day 22 through 41 delayed vaginal opening by 4 days. Male rats exposure to DACT, DEA, or DIA during postnatal days 23-53 were found to show preputial separation and decreases in prostate weights.

E. **Altered Pregnancy Maintenance**

It is well known that the hormonal requirements of the corpus luteum (CL) change as the rat progresses through different stages of gestation. After the first week of gestation, the CL no longer requires prolactin for support and becomes dependent on LH during gestation days (GD) 7-16. During this time as little as 2-4 hours deprivation of LH may be sufficient to terminate pregnancy. **Atrazine** and the metabolites **2-hydroxyatrazine, DACT**, **DEA**, and **DIA** have been reported to alter pregnancy maintenance. These compounds have been found to induce full litter resorption, induce pseudopregnancy, prevent pre or post-implantation, and/or delay parturition. Although **2-hydroxyatrazine** has been shown to alter pregnancy and delay puberty in males, it has not been found to induce mammary gland tumors. Therefore, based on the absence of mammary gland tumor induction by 2-hydroxyatrazine and inconclusive data that show 2-hydroxyatrazine's effect on the LH surge and/or LH-dependent events, the WOE **does not** support including it in the common mechanism group at this time.

VI. Conclusions and Final Grouping of the Candidate Group Compounds Based on a Common Mechanism of Action

To satisfy the requirements of the Food Quality Protection Act of 1996 to assess the cumulative effects of chemicals that have a common mechanism of toxicity, OPP has determined that some of the candidate group compounds can be grouped based on a common mechanism of toxicity. Based upon the weight-of-evidence provided in studies by registrants and EPA laboratories and in studies reported in the literature, the pesticides **atrazine, propazine, simazine, and metabolites diaminochlorotriazine (DACT), desisopropyl s-atrazine (DIA), and desethyl s-atrazine (DEA)** should be considered as a **Common Mechanism Group**, based on suppression of the LH ovulatory surge and the consequent effects on reproductive function and development. Several compounds were excluded (including Express and 2-hydroxyatrazine) from this grouping on the basis of not having sufficient similarity to the remaining compounds with respect to metabolism, pharmacokinetics, and toxic effects (i.e., mammary gland tumors and/or neuroendocrine effects). Others were excluded because they were no longer a registered compound with the US EPA, had minimal human exposure, or were registered outside the United States. Following the initiation of a cumulative risk assessment, the Common Mechanism Group may be modified as a result of the review of new or existing data.

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